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(57) Abstract

A compound comprising a superoxide oxidant or reductant to which is directly or indirectly linked an NO or NO_2 group. More particularly, compounds having the formula: D-X-R, wherein R is a moiety that oxidizes and/or reduces superoxide to oxygen and/or hydrogen peroxide under physiological conditions; X is S, N, O or C; and D is NO or NO_2. R can be a functionality containing an unpaired electron, a cation such as a physiologically acceptable metal ion, hydrogen or a protective group or R can be a complex of a transition metal and a macrocyclic ligand that dismutes superoxide under physiological conditions. These compounds can be used alone or in combination or concurrently with other therapeutic agents, particularly nitric oxide adducts. Further, the invention provides that the superoxide oxidants or reductants which have not been linked to an NO or NO_2 group can be administered in combination or concurrently with nitric oxide or nitric oxide adducts. They are useful for preventing superoxide cell damage and for treating inflammatory disorders in mammals, particularly humans.

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NITROSYLATED AND NITRATED SUPEROXIDE OXIDANTS AND REDUCTANTS

The invention relates to novel nitroso and nitro derivatives of compounds which oxidize and/or reduce superoxide either catalytically or stoichiometrically and to pharmaceutical compositions comprising the nitroso or nitro derivative of the invention together with a pharmaceutically acceptable carrier. The invention also relates to their use in treating a variety of disorders.

During cellular metabolism, a variety of toxic oxygen-related species such as hydroxyl radical (•OH); hydrogen peroxide (H_2O_2); and superoxide (O_2)· are produced. Left unchecked, these free radical species damage cells. However, cells have systems to rid themselves of these metabolic by-products. For example, superoxide dismutase (SOD) converts superoxide to O_2 and H_2O_2, and the enzymes catalase, glutathione peroxidase, and glutathione transferase either alone, or in some cases with cofactors, have the ability to convert the H_2O_2 to H_2O.

The superoxide dismutases are used as pharmacological agents. They are applied to the treatment of inflammatory diseases and the reperfusion injury associated with skin grafts, organ transplants, frostbite and myocardial infarction.

Possibly the most important source of mutagenic alterations in DNA is oxidative damage. Excited-oxygen species such as hydrogen peroxide, hydroxyl radicals, and superoxide radicals arise during irradiation or as a byproduct of aerobic metabolism. Cells possess an elaborate defense system to destroy these reactive species, including enzymes such as catalase and superoxide dismutase.

There are essentially three categories of superoxide dismutases. The manganese-containing superoxide dismutases (MnSODs) are found in prokaryotes and in the matrix of mitochondria. The related iron-containing superoxide dismutases (FeSODs) are found in prokaryotes and in a few families of plants. The unrelated copper and zinc superoxide dismutases (CuZnSODs) occur primarily in the cytosol of eukaryotic cells and in chloroplasts but have also been found in a few species of bacteria.

Amino acid sequences indicate that MnSODs and FeSODs from diverse sources are closely related, whereas CuZnSODs are not structurally similar. However, all of these SODs catalyze the same process and do so with comparable efficiency.

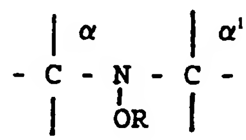
Most MnSODs and FeSODs are active only with the native metal at the active site. All SODs described to date are multimeric. The cytosolic CuZnSODs, as well as most prokaryotic MnSODs and FeSODs, are homodimers while the

MnSODs from mitochondria and from certain thermophilic bacteria are homotetramers. Mammalian extracellular fluids contain a tetrameric glycosylated CuZnSOD.

To realize the beneficial antioxidant activity of superoxide dismutase without the difficulties that attend the administration and use of these large and labile enzymes, efforts have also been addressed to identifying relatively low molecular weight compounds that "mimic" the superoxide detoxification activity and antioxidant effect of SOD while possessing greater *in vivo* stability and the efficacy of convenient forms of administration.

A group of stable nitroxide free radicals have been shown to possess metal-independent superoxide dismutase-like activity. Unlike SOD, these compounds are of relatively low molecular weight and readily penetrate into the cell.

Nilsson and Bylund-Fellenius, WO 88/05044, discloses compounds having the formula:



wherein R is an unpaired electron, a cation such as a physiologically acceptable metal ion, hydrogen or a protective group; and the α and α' carbons are substituted such that when R is an unpaired electron the compound is a stable nitroxide (nitroxyl radical). Preferred are compounds where the α and α' carbons are substituted to form 5- or 6-membered ring structures. They are useful for preventing and treating ischemic cell damage in mammals.

Examples of these nitroxides are 4-hydroxy-2,2,6,6-tetramethyl-piperidinyloxyl (TEMPOL) and 4-amino-2,2,6,6-tetramethyl-piperidinoxyl(TEMPAMINE). See, for example, An and Hsie, 1994; Hahn *et al.*, 1994; An and Hsie, 1993; Reddan *et al.*, 1993; Riley *et al.*, 1993; An and Hsie, 1992; DeGraff *et al.*, 1992a; DeGraff *et al.*, 1992b; Reddan *et al.*, 1992; Bonne *et al.*, 1991; Gelvan *et al.*, 1991; Mitchell *et al.*, 1991; Pogrebniak *et al.*, 1991; Samuni *et al.*, 1991; Mitchell *et al.*, 1990; and Samuni *et al.*, 1990.

Mitchell *et al.*, WO 91/13619 (1991) discloses compositions containing a metal independent nitroxide compound. Mitchell *et al.*, 1990, reports SOD mimetic activity in spiro[cyclohexane-1,2'-(4',4'-dimethyloxazolidine-3'-oxyl)](CHD) and 2-ethyl-2,4,4-trimethyl oxazolidine-3-oxyl(OXANO). Jarabak and Harvey, 1993, and Samuni *et al.*, 1991, report SOD activity in 3-carboxy-2,2,5,5-tetramethylproxyl(PCA).

Fridovich *et al.*, U.S. Patent No. 5,227,405 discloses superoxide dismutase-like activity in certain manganese desferroxamine complexes. Riley *et al.*, 1994, reports SOD mimetic activity for other manganese macrocyclic ligand complexes, including the Mn(II) complex of the macrocyclic ligand, 1,4,7,10,13-pentaaxacyclopentadecane.

Baudry *et al.*, 1993, report SOD mimetic activity in several salen-manganese complexes, including both neutral and cationic complexes. Kitajima *et al.*, 1993, reports monomeric(benzoato) manganese (II) SOD mimetic complexes, such as Mn(OBz)(hydroxytris(3,5-diisopropyl-1-pyrazolyl)borate and Mn(Obz) (3,5-diisopropyl-pyrazole)(hydroxytris(3,5-diisopropyl-1-pyrazolyl)borate.

Schechinger *et al.*, 1988, reports SOD mimic activity in a Cu(II) desferritiocin complex.

SOD-like activity has also been observed in diSchiff base coordinated copper complexes. Nagele *et al.*, 1994, reports such activity in copper-putrescine-pyridine[(Cu-Pu(Py)$_2$]. Meisel *et al.*, 1990, reports such activity in another diSchiff base, CuPu(Im)$_2$. Linss and Weser, 1986, reports SOD mimetic activity in copper complexes of the diSchiff bases of pyridine-2-aldehyde and 1,4-diaminobutane. Duran *et al.*, 1993, reports the SOD mimetic activity of copper(II)(3,5-diisopropylsalicylate)$_2$ (CuDIPS). Pellitier *et al.*, 1993, reports the SOD mimetic activity of tetrakis(3,5-diisopropylsalicylato)dicopper [Cu(II)$_2$(3,5-DIPS)$_4$]. Yaping *et al.*, 1992, reports copper complex SOD mimetics that are 20-membered macrocyclic bicopper(II) complexes and 13-membered macrocyclic dioxotetramine copper(II) complexes, respectively. They are more active in scavenging reactive oxygen species than polyamine Cu(II)-Zn(II) complexes and copper(II) complexes of bis-Schiff bases(diSchiff bases). Foye, 1992, reports copper complex SOD mimetics that are copper complexes of quinolinium and pyridinium bis(methylthio) and methylthio amino derivatives. Haseloff *et al.*, 1992, reports SOD mimetic activity in Cu(II)$_2$(indomethacin)$_4$.

Other SOD mimetics that have been identified are metal(including Cu^+, Fe^{2+} and Mn^{2+}) complexes of pyrimines. For example, Itami *et al.* reports a complex containing Fe^{2+} and L-2(2-pyridyl)-1-pyrroline-5-carboxylic acid. Weiss *et al.*, 1993, identified metal complexed SOD mimetics exemplified by Fe(III)-tris[N-(pyridylmethyl0-2-aminoethyl]amine(Fe-TPAA) and Fe(II)-tetrakis-N,N',N,N'-(pyridylmethyl)ethylenediamine(Fe-TPEN), respectively.

The NO and NO_2 adducts of the current invention provide a number of interrelated advantages. The use of NO-SOD, NO_2 -SOD, and nitrosylated as well as nitrated compounds which possess the ability to detoxify superoxide by catalytic or non-catalytic redox mechanisms enhances the effectiveness of NO therapy because the SOD or compound with SOD-like activity eliminates superoxides which would otherwise consume the administered NO, particularly in its free charged and uncharged species. Nitric oxide and SOD or compounds with SOD-like activity each interact with superoxide, producing peroxynitrite and hydrogen peroxide, respectively. The degradation products of peroxynitrite and hydrogen peroxide can each be toxic depending on the location and conditions under which they are formed, particularly as their concentration increases. Since NO● and SOD species both interact with superoxide, lower concentrations of each of peroxynitrite and hydrogen peroxide are produced by any given amount of superoxide, thereby limiting any nitric oxide-related toxicity. Further, the adducts of the invention make it possible to deliver nitric oxide in a non-toxic form. In addition, their use enhances the effectiveness of superoxide scavenging by combining nitric oxide inactivation of superoxide with protection of the nitric oxide by the compounds with SOD-like activity.

The present invention provides a compound comprising a superoxide oxidant or reductant to which is directly or indirectly linked an NO or NO_2 group. More particularly the invention provides compounds having the formula:

D-X-R

wherein R is a moiety that oxidizes and/or reduces superoxide to oxygen and/or hydrogen peroxide under physiological conditions; X is S, N, O or C, and D is NO or NO_2. For example, R can be a superoxide dismutase enzyme.

Alternatively, R can be a functional group with an unpaired electron, such as a nitroxide. R can also be a complex of a transition metal and a macrocyclic ligand that dismutes superoxide under physiological conditions.

The invention further provides a compound comprising a superoxide oxidant or reductant to which is directly or indirectly linked (i) an NO or NO_2 group and (ii) a specific binding ligand. Preferably, the specific binding ligand is specifically bindable with a cell surface or extracellular matrix specific binding partner. It is also preferred that the superoxide oxidant or reductant is linked to the NO or NO_2 group through the specific binding ligand.

The compounds of the invention are useful for preventing and treating ischemic cell damage in mammals, particularly humans.

In another aspect the invention provides a method of protecting cells from superoxide radical-induced toxicity by contacting the cells with a protective amount of the compounds of the invention.

It also provides a method for treating an inflammatory condition with such compounds particularly where such inflammatory condition is of the lung and administration is to the lung. It also provides a method of treating a disorder resulting from aberrant smooth muscle or other function by such administration of the compounds of the invention.

In another aspect, the invention provides for combined or concurrent therapies by administering a superoxide dismutase, superoxide dismutase mimetic or other compound that oxidizes and/or reduces superoxide to oxygen and/or

hydrogen peroxide in combination with a nitric oxide adduct. This aspect further provides methods and pharmaceutical preparations comprising such nitric oxide adducts and compounds of the invention.

Figure 1 illustrates a synthesis pathway for preparation of a compound of formula IA.

Figure 2 illustrates a synthesis pathway for preparation of a compound of formula IIA.

Figure 3 illustrates a synthesis pathway for preparation of a compound of formula IIIA.

Figure 4 illustrates a synthesis pathway for preparation of a compound of formula IVA.

Figure 5A illustrates a synthesis pathway for preparation of a compound of formula VA.

Figure 5B illustrates a synthesis pathway for preparation of a compound of formula VB.

Figure 6 illustrates a synthesis pathway for preparation of a compound of formula VIA.

Figure 7 illustrates a synthesis pathway for preparation of a compound of formula VIIA.

As summarized above, the invention provides a compound comprising a superoxide oxidant or reductant to which is directly or indirectly linked an NO or NO_2 group.

One embodiment of the invention provides compounds having the formula:

D-X-R

wherein R is a moiety that oxidizes and/or reduces superoxide to oxygen and/or hydrogen peroxide under physiological conditions; X is S, N, O or C; and D is NO or NO_2. R can be for example, any of the superoxide dismutase enzymes or mimetics.

The term "lower alkyl" as used herein refers to a branched or straight chain alkyl groups comprising one to ten carbon atoms, including methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, neopentyl and the like.

The term "alkoxy" as used herein refers to R-O- wherein R is lower alkyl as defined above. Representative examples of alkoxy groups include methoxy, ethoxy, t-butoxy and the like.

The term "alkoxyalkyl" as used herein refers to an alkoxy group as previously defined appended to an alkyl group as previously defined. Examples of alkoxyalkyl include, but are not limited to, methoxymethyl, methoxyethyl, isopropoxymethyl and the like.

The term "amino" as used herein refers to $-NH_2$.

The term "dialkylamino" as used herein refers to $(R_{12})N$- wherein each R_{12} is independently a lower alkyl, for example dimethylamino, diethylamino, methyl propylamino and the like.

The term "nitro" as used herein refers to the group $-NO_2$.

The term "nitroso" as used herein refers to the group -NO.

The term "hydroxyl" or "hydroxy" as used herein refers to the group -OH.

The term "cyano" as used herein refers to the group -CN.

The term "carbamoyl" as used herein refers to $H_2N-C(O)O-$.

The term N,N-dialkylcarbamoyl as used herein refers to $R_{12}(R_{12})N-C(O)O-$ wherein each R_{12} is independently a lower alkyl, for example dimethylamino, diethylamino, methyl propylamino and the like.

The term N-alkylcarbamoyl as used herein refers to $R_{12}HN-C(O)O-$ wherein R_{12} is a lower alkyl, for example methylamino, ethylamino, propylamino and the like.

The term "aryl" as used herein refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. Aryl groups (including bicyclic aryl groups) can be unsubstituted or substituted with one, two or three substituents independently selected from the group consisting of lower alkyl, haloalkyl, alkoxy, amino, alkylamino, dialkylamino, hydroxy, halo, and nitro. In addition, substituted aryl groups include tetrafluorophenyl and pentafluorophenyl.

The term "arylthio" as used herein refers to $R_{13}S-$ wherein R_{13} is aryl.

The term "alkanoyl" as used herein refers to $R_{12}C(O)-$ wherein R_{12} is a lower alkyl.

The term "carboxyl" as used herein refers to the group -COOH.

The term "guanidino" as used herein refers to $H_2N-C(=NH)NH-$.

The term "arylakyl" as used herein refers to a lower alkyl radical to which is appended an aryl group. Representative arylalkyl groups include benzyl, phenylethyl, hydroxybenzyl, fluorobenzyl, fluorophenylethyl and the like.

The term "cycloalkyl" as used herein refers to an alicyclic group comprising from 3 to 7 carbon atoms including, but not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like.

The term "halogen" or "halo" as used herein refers to I, Br, Cl, or F. The term "haloalkyl" as used herein refers to a lower alkyl radical, as defined above, bearing at least one halogen substituent, for example, chloromethyl, fluoroethyl or trifluoromethyl and the like.

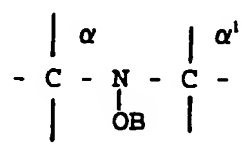
The term "heteroaryl" as used herein refers to a mono-or bi-cyclic ring system containing one or two aromatic rings and containing at least one nitrogen, oxygen, or sulfur in an aromatic ring. Heteroaryl groups (including bicyclic heteroaryl groups) can be unsubstituted or substituted with one, two, or three substituents independently selected from the group consisting of lower alkyl, haloalkyl, alkoxy, amino, alkylamino, dialkylamino, hydroxy, halo and nitro. Examples of heteroaryl groups include, but are not limited to, pyridine, pyrazine, pyrimidine, pyridazine, pyrazole, triazole, thiazole, isothiazole, benzothiazole, benzoxazole, thiadiazole, oxazole, pyrrole, imidazole, and isoxazole.

The term "heterocyclic ring" refers to any 3-, 4-, 5-, 6- or 7-membered nonaromatic ring containing at least one nitrogen atom which is bonded to an atom which is not part of

the heterocyclic ring. In addition, the heterocyclic ring may also contain one additional heteroatom which can be nitrogen, oxygen, or sulfur.

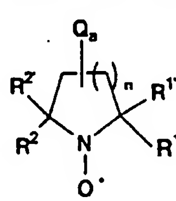
Compounds of the invention which have one or more asymmetric carbon atoms can exist as the optically pure enantiomers, pure diastereomers, mixtures of enantiomers, mixtures of diastereomers, racemic mixtures of enantiomers, diastereomeric racemates or mixtures of diastereomeric racemates. It is to be understood that the present invention anticipates and includes all such forms within its scope.

The invention also provides nitrosylated and nitrated derivatives of compounds having the formula:



wherein B is an unpaired electron, a cation such as a physiologically acceptable metal ion, hydrogen or a protective group; and at least one of the α and α^1 carbons is directly or indirectly linked to an NO or NO_2 group. Preferred are compounds where the α and α^1 carbons are substituted to form 5- or 6-membered ring structures.

A preferred embodiment of the invention relates to nitroso and nitro derivatives having the formula:



I

wherein

n is an integer from 0 to 4;

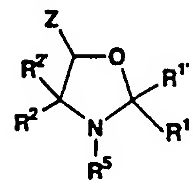
R^1, $R^{1'}$, R^2 and $R^{2'}$ are each independently selected from the group consisting of:

- (i) $-(CH_2)_a$-X-D, in which a is an integer from 1 to 4 and X is S, O, C, N(CO)R^o or NR^o, in which R^o is H, alkyl, cycloalkyl, aryl, heteroaryl, or heterocyclic ring system, and D is NO or NO_2;
- (ii) alkyl;
- (iii) $-(CH_2)_aR^3$, in which a is as defined above and R^3 is cycloalkyl, aryl, heteroaryl, or heterocyclic ring system;
- (iv) $-(CH_2)_aOR^4$, in which a is as defined above, and R^4 is cycloalkyl, alkyl, aryl, heteroaryl, or heterocyclic ring system;
- (v) $-(CH_2)_aNHR^4$, in which a and R^4 are as defined above; or in which, when taken together, one or both of R^1 and $R^{1'}$ or R^2 and $R^{2'}$ are selected from the group consisting of cycloalkyl, norbornyl and cholestane; and

Q_a, in which a is as defined above, is located on the nitrogen-containing ring at one or more positions not substituted by R^1, $R^{1'}$, R^2, or $R^{2'}$ and is selected from the group consisting of:

- (i) $-(CH_2)_b$-X-D, in which b is an integer from 0 to 4 and X and D are as defined above;
- (ii) -C(O)X-$(CH_2)_c$-X-D, in which c is an integer from 2 to 6 and X and D are as defined as above;
- (iii) -X-[C(O)C($(CH_2)_a$-X-D)-NH]$_a$p' wherein p' is a nitrogen protecting group and X, D, and a are defined as above; and
- (iv) H, with the proviso that at least one of R^1, $R^{1'}$, R^2 or $R^{2'}$ consists of $-(CH_2)_a$-X-D; in which a, X and D are as defined above.

Another embodiment provides nitroso derivatives having the formula:



II

wherein R^1, $R^{1'}$, R^2, and $R^{2'}$ are as defined above;
Z is selected from the group consisting of:

(i) $-(CH_2)_a-X-D$, in which a, X and D are as defined above;

(ii) $-C(O)X-(CH_2)_c-X-D$, in which c, X and D are as defined above; and

(iii) H, with the proviso that at least one of R^1, $R^{1'}$, R^2, or $R^{2'}$ consists of $-(CH_2)_a-X-D$, in which a, X and D are defined as above; and

R^5 is selected from the group consisting of O● and OH.

Another embodiment provides nitrosylated compounds having the formula:

D-X-R

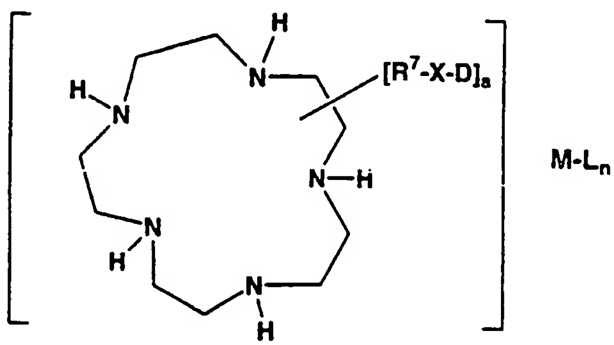
wherein R is a complex of a transition metal and a macrocyclic ligand, which complex converts superoxide to oxygen and/or hydrogen peroxide under physiological conditions; X is S, N, O or C; and D is NO or NO_2.

Another embodiment provides nitrosylated compounds having the formula:

$$R^6-[R^7-X-D]_a$$

wherein R^6 is a complex of a transition metal and a macrocyclic ligand that dismutes superoxide under physiological conditions; R^7 is a bond or a linking group; and a, X and D are defined as above.

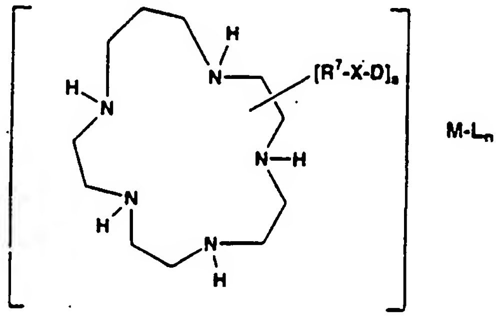
Another embodiment provides compounds having the formula:



III

wherein M is iron, copper or manganese, L is a suitable organic or inorganic ligand or organic or inorganic charge neutralizing anion which is derived from any monodentate or polydentate coordinating ligand or ligand system or the corresponding anion thereof (for example acetic acid acetate anion, alcohol or alkoxide anion); and R^7, a, n, X and D are as defined above.

Another embodiment provides compounds having the formula:

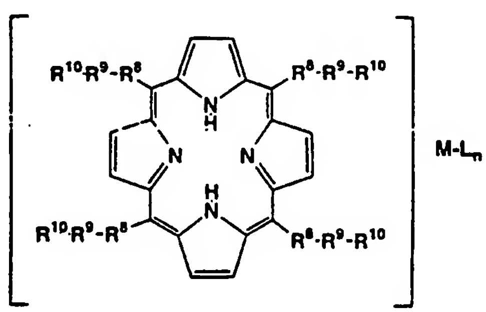


IV

wherein M, L, R^7, a, n, X and D are as defined above.

Another embodiment provides such compounds wherein the macrocyclic ligand is a porphyrin, such as tetrakis(4-benzoic acid) porphyrin.

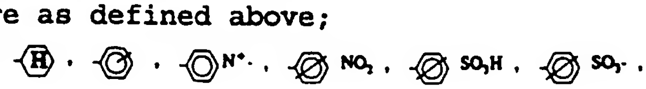
Another embodiment provides compounds of the formula:



V

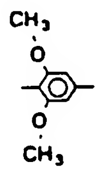
or a pharmaceutically acceptable salt thereof, wherein:

M, L and n are as defined above;

R^8 is a bond, 

 U  V wherein U is a halogen and V is an alkyl group and wherein  indicates bonding to R^9 at any position and  indicates bonding to R^9 and the substituent at any position; and

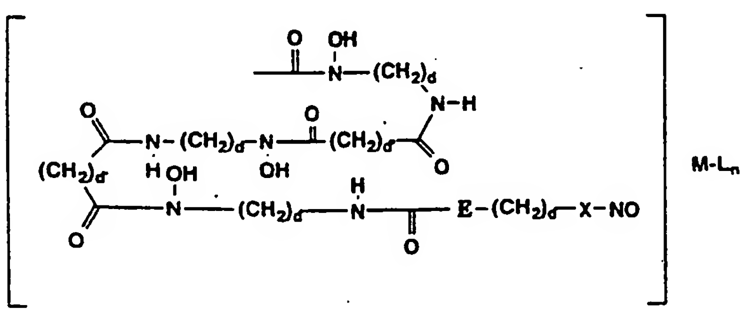
R^9 is a bond, $-(CY'_2)_m-$, $-(CY'_2-CY'=CY')_m-$, $-(CY'_2-CY'_2-CH=CH)_m-$ or $-(CY'_2-C)_m-$, wherein Y' is hydrogen or an alkyl group and wherein m is 1 to 8; or

 ; and

R^{10} is -X-D, $-CH_2-CH_2-\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{\overset{|}{\underset{|}{C}}}}-X-D$, $-(CH_2)_{1-3}-X-D$, -NH-NO or NO^--NO,

in which X R^0. and D are defined as above.

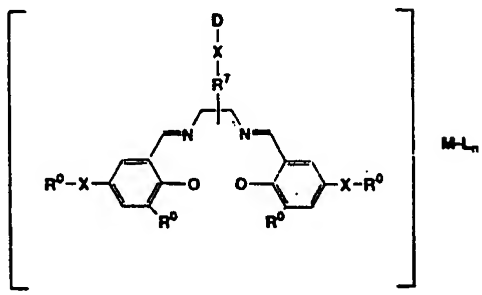
Another embodiment provides compounds having the formula:



VI

wherein d and d' are each independently an integer from 1 to 6 and E is a covalent bond, S, N, O, or -C-N-(CO)R^0 wherein M, LR^0 and X are as defined above.

Another embodiment provides compounds having the formula:



VII

wherein M, R^7, L, a, R^0 and X are defined as above.

Another embodiment of the invention provides a compound comprising a superoxide oxidant or reductant to which is directly or indirectly linked (i) an NO or NO_2 group and (ii) a specific binding ligand. Preferably, the specific binding ligand is specifically bindable with a cell surface or extracellular matrix specific binding partner. It is also preferred that the superoxide oxidant or reductant is linked to the NO or NO_2 group through the specific binding ligand.

Preferred among the compounds of this embodiment are those wherein the specific binding ligand is selected from the group consisting of:

$(NH\text{-}CH_2\text{-}CH_2\text{-}NH)_n\text{-}H$ where n = 1-6;

NH_2-His Arg His His Pro Arg Glu Met Lys Lys Arg Val Glu Asp Leu-COOH;

NH_2-Arg Glu His Ser Glu Arg Lys Lys Arg Arg Arg Glu Ser Glu Cys Lys Ala Ala-COOH;

NH_2-Arg Glu His Ser Glu Arg Lys Lys Arg Arg Arg-COOH

NH_2-Arg Glu His Ser Glu Arg Lys Lys Arg Arg Ala-COOH;

NH_2-Arg Glu His Ser Glu Arg Lys Lys Arg Arg Arg Ala Ser Glu Cys Lys Ala Ala-COOH;

NH_2-Arg Glu His Ser Glu Arg Lys Lys Arg Arg Arg Glu Ser Glu Cys Ala Ala Ala-COOH;

NH_2-Arg Glu His Ser Glu Arg Lys Lys Arg Arg Arg Ala Ser Ala Cys Lys Ala Ala COOH;

NH_2-Arg Glu His Ser Glu Arg Lys Lys Arg Arg Arg Ala Ser Glu Cys Ala Ala Ala-COOH;

NH_2-Arg Glu His Ser Glu Arg Lys Lys Gly Arg Arg Ala Ser Glu Cys Ala Ala Ala-COOH; and

$(Arg)_n$, $(Lys)_n$, $(Arg)_n$, $(Lys)_n$, $(Arg\ Lys)_n$, $(Lys\ Arg)_n$, and $(Lys\ Lys\ Arg\ Arg)_n$, wherein n is 1 to 12, any of which can be substituted with at least one Cys.

Compounds of the invention which have one or more asymmetric carbon atoms may exist as the optically pure enantiomers, pure diastereomers, mixtures of enantiomers, mixtures of diastereomers, racemic mixtures of enantiomers, diastereomeric racemates or mixtures of diastereomeric racemates. The present invention contemplates and includes within its scope all such isomers and mixtures thereof.

The nitrosylation of proteins is described in detail in WO93/09806, published 27 May 1993, and the methods of protein nitrosylation described and exemplified there are also applicable to the superoxide dismutases in accordance with the present invention. Mono S-nitrosylation is best achieved by incubating peptides and proteins (in deionized water in an equimolar concentration of acidified nitrite (final concentration 0.5 N HCl) for a period of 1-30 minutes. The incubation time depends on the efficiency of nitrosation and the tolerance of the protein. Nitrosation can also be achieved with a variety of other nitrosating agents including compounds such as S-nitroso-cysteine, S-nitroso-glutathione and related alkyl nitrites. These compounds are to be used when the peptide or protein does not tolerate harsh acidic conditions.

With reference to the aspect of the invention relating to nitrosylated superoxide dismutase proteins themselves it is particularly preferred polynitrosylated. Synthesis of polynitrosated peptides and proteins can be achieved in several ways.

There are two principal ways of achieving poly S-nitrosation. In the first, the peptide or protein is reduced in 100-1000 molar excess dithiothreitol for 30-60 minutes. This exposes intramolecular thiols. The peptide or protein is separated from dithiothreitol by gel filtration (G-25). The protein is then exposed to increasing concentrations of acidified nitrite (0.5 N HCl) in relative excess over protein. Complementary measurements of Saville indicate when S-nitrosation is complete. For example, with albumin, this procedure leads to approximately 20 intramolecular S-NO derivatives.

Alternatively, the protein can be treated with thiolating agent such as N-acetyl homocysteine thiolactone. This tends to add homocystine groups to exposed amine residues in proteins. The derivatized protein can then be S-nitrosated by exposure to acidified nitrite. Exposure to increasing concentrations of nitrite with complementary measurements of Saville can be used to ascertain when S-nitrosation is maximal. Alternatively, thiol groups can be quantified on the protein using standard methodologies and then the protein treated with a stoichiometric concentration of acidified nitrite (0.5 N HCl).

Polynitrosation of nucleophilic functional groups (other than thiol) can be achieved when proteins are incubated with excess acidified nitrite. The order of protein reactivity is tyrosine followed by amines on residues such as trytophan. Amide linkages are less reactive. Accordingly, many NO groups can be added to proteins by simply incubating the protein with high excess acidified nitrite. These experiments are performed in 0.5 normal HCl with incubations of approximately one hour. ^{15}N NMR can be used to determine where the addition (or substitution) by NO takes place.

Further, nitrosation can be achieved by exposure to authentic nitric oxide gas under anaerobic conditions in the presence of trace quantities of metals such as copper or silver ion. For successful nitrosation proteins should be incubated in at least 5 atmospheres of NO gas for several hours. Incubation time is protein specific. This can lead to NO attachment to a variety of protein bases. Best characterized reactions involve primary amines. This mechanism provides a pathway to sustain N-nitrosation reactions without deamination. Specifically, exposure to acidified nitrite would otherwise lead to deamination of primary amines whereas this method leads to formation of N-

hydroxynitrosamines with potent bioactivity. Similar substitutions at other basic centers also occur. These and other common methods to effect nitrosation are described in the literature, *c.f.*, D.H.L. Williams, Nitrosation, Cambridge University Press, Cambridge (1988).

Some of the compounds of the present invention may be synthesized by the reaction schemes shown in Figures 1 through 7 in which R-R^{11} are as defined above or as depicted in the reaction schemes for formulas **IA** through **VIIA**; P^1 is a nitrogen protecting group; and P^2 is a sulfur protecting group.

The reactions are performed in solvents appropriate to the reagents and materials employed and are suitable for the transformations being effected. It is understood by those skilled in the art of organic synthesis that the functionality present on the heterocyclic ring and other portions of the molecule must be consistent with the chemical transformation proposed. This will, on occasion, necessitate judgment by the routineer as to the order of synthetic steps, protecting groups required, and deprotection conditions. Substituents on the starting materials may be incompatible with some of the reaction conditions required in some of the methods described, but alternative methods and substituents compatible with the reaction conditions will be readily apparent to skilled practitioners in the art. The use of sulfur and nitrogen-protecting groups is well known in the art for protecting thiol and amino groups against undesirable reactions during a synthetic procedure and many such protecting groups are known, c.f., T.H. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*, John Wiley & Sons, New York (1991).

Nitroxide compounds of formula I wherein R^1 $R^{1'}$, R^2, $R^{2'}$ and Q are as defined above can be prepared according to the reaction scheme depicted in Figure 1, in which a substituted piperidine is representative of the saturated nitrogen-containing heterocyclic ring as defined above. 2,2,6,6-Tetramethyl-4-piperidinol with the ring nitrogen protected, for example as the t-butoxycarbonyl (t-Boc) derivative, is converted to a thiol of formula 2 using a suitable hydroxyl activating agent such as paratoluenesulfonyl chloride or trifluoromethanesulfonic anhydride in the presence of a base (such as triethylamine), followed by treatment with a suitable sulfur nucleophile such as sodium hydrosulfide. The thiol compound of formula **2** is, in turn, protected as a thiocarbamate by reacting it with a suitable isocyanate such as methoxymethyl isocyanate to afford the compound of formula 3. Deprotection of the ring nitrogen of the compound of formula 3 (t-butoxycarbonyl groups are typically cleaved by treatment with a strong acid such as trifluoroacetic acid) and then oxidizing the resulting amine by treatment with a mild oxidizing agent such as 3-chloroperoxybenzoic acid or sodium tungstate in the presence of aqueous hydrogen peroxide affords the compound of formula **4**. The thiol of the compound of formula **4** is deprotected to afford the compound of formula **5**. N-methoxymethyl-carbamates are typically cleaved by hydrolysis with a solution of aqueous base. The thiol moiety is then nitrosated using a suitable mild nitrosylating agent such as sodium nitrite in aqueous hydrochloric acid to afford a nitrosothiol of formula **IA**.

Nitroxide compounds of formula II wherein R^1, $R^{1'}$, R^2 $R^{2'}$, and Z are defined as above can be prepared according to the reaction scheme depicted in Figure 2, in which a five membered substituted oxazolidine-1-oxyl is representative of the saturated nitrogen-containing heterocyclic ring as

defined above. A compound of formula 6 with the thiol moiety protected, for example as the S-(N-methoxymethylcarbamate), wherein a and R^1 are defined as above, is converted to the compound of formula 7 by reacting it with 2-amino-2-methyl-1-propanol in a suitable solvent such as benzene and heating to reflux in the presence of a suitable acid catalyst such as paratoluenesulfonic acid with the azeotropic removal of water. The oxazolidine of formula 7 is converted to the oxazolidine-1-oxyl of formula 8 by treatment with a mild oxidizing agent such as 3-chloroperoxybenzoic acid or sodium tungstate in the presence of aqueous hydrogen peroxide. The compound of formula 8 is then treated with a suitable reagent for removing the thiol protecting group. Treatment with mild aqueous base is the preferred method for removing a S-(N-methoxymethyl-carbamate group. The thiol is then nitrosylated with a mild nitrosating agent, for example, by treatment with sodium nitrite in aqueous hydrochloric acid to afford the compound of formula **IIA**.

Azamacrocycles of formula **III**, wherein R^7, M, D, a, X, and L are as defined above, and L^1 and L^2 are independently selected from L can be prepared according to the reaction scheme depicted in Figure 3, in which manganese complexes of nitrogen-containing fifteen-membered macrocyclic ligands containing one or two nitrosylated thiols are representative of the transition metal complexes as defined above. A thiol-containing α-amino acid of formula 9 with the nitrogen and sulfur moieties protected, wherein a, P^1, and P^2 are defined as above, is activated at the carboxylate with a suitable activating agent such that, when treated with ethylene diamine, it forms the amide of formula 10. Preferred protecting groups for the α-amino acid of formula 9 are a t-Boc for the amine and a benzyl group for the thiol. For example, one method of activation is formation of the mixed anhydride by reaction of the acid with isobutylchloroformate

in the presence of a base such as N-methylmorpholine and subsequent addition of the amine nucleophile. The amide of formula **10** is converted to the triamine of formula **11** by removing the nitrogen protecting group and then reducing the amide functionality. For example, cleavage of a t-butoxycarbamate group is accomplished with a strong acid such as trifluoroacetic acid in methylene chloride or 4N hydrochloric acid in dioxane. The amide is then reduced with a reducing agent such as lithium aluminum hydride or borane in an aprotic solvent such as ether or THF. The compound of formula **11** is then converted to the compound of formula **12** by treatment with excess paratoluenesulfonyl chloride in the presence of a non nucleophilic base such as triethylamine or pyridine. Treatment of a compound of formula **12** with a strong base such as sodium hydride in an anhydrous aprotic solvent such as THF or DMF to form the dianion and then reacting the dianion with ethylene carbonate affords a compound of formula **13**. Activation of the hydroxyl moieties in a compound of formula **13** with a suitable hydroxyl activating agent such as paratoluenesulfonyl chloride or trifluoromethanesufonic anhydride in the presence of a base (such as triethylamine) followed by treatment with a dianion of formula **14** wherein R^{11} is H, or R^0, or $-(CH_2)_a-S-P^2$, wherein R^0, a, and P^2 are defined as above affords a compound of formula **15**. The compound of formula **15** is converted to the compound of formula **16** by the removing the amine and sulfur protecting groups. Paratoluenensulfonamides and thiobenzyl ethers can be cleaved with sodium in liquid ammonia. Nitrosation of the thiol group(s) in a compound of formula **16** using a stoichiometric amount of suitable mild nitrosating agent such as sodium nitrite in aqueous hydrochloric acid or t-butyl nitrite in chloroform affords a compound of formula **17**. The compound of formula **17** is then converted to the complex of formula **IIIA** by reaction with a suitable manganese II compound under essentially anaerobic conditions.

Azamacrocycles of formula IV wherein R^7, M, D, a, X, L^1, and L^2 are defined as above, can be prepared according to the reaction scheme depicted in Figure 4, in which manganese complexes of nitrogen-containing sixteen-membered macrocyclic ligands containing one or two nitrosylated thiols are representative of the transition metal complexes as defined above. Activation of the hydroxyl moieties in a compound of formula 13 with a suitable hydroxyl activating agent such as paratoluenesulfonyl chloride or trifluoromethanesufonic anhydride in the presence of a base (such as triethylamine), followed by treatment with a dianion of formula 18 wherein R^{11} is as defined as above, affords a compound of formula **19**. The compound of formula 19 is converted to the compound of formula 20 by the removing the amine and sulfur protecting groups. For example, paratolunensulfonamides and thiobenzyl ethers can be cleaved with sodium in liquid ammonia. Nitrosation of the thiol group(s) in a compound of formula 20 using a suitable mild nitrosating agent such as a stoichiometric quantity of sodium nitrite in aqueous hydrochloric acid or t-butyl nitrite in chloroform affords a compound of formula 21. The compound of formula 21 is then converted to the complex of formula IVA by reaction with a suitable manganese II compound under essentially anaerobic conditions.

Azamacrocycles of formula V in which M, L, R^8, R^9, and R^{10} are defined as above can be prepared according to the reaction scheme depicted in Figure 5, in which manganese complexes of porphyrin rings are representative of the transition metal complexes as defined above and wherein w is OH, SH, NH_2 or NHR° and x, c, and p^2 are defined as above.

Starting with compound 22, the w groups thereof are reacted with a compound of the formula 23, wherein T is an activated carbonyl-containing substituent selected from the

group consisting of a mixed anhydride, an acid chloride, an isocyanate, or a chloroformate, and c and P^2 are defined as above, to afford compound **24**. The thiol protecting group is removed (aqueous base is used to remove S-(N-methoxymethyl-carbamate) groups) to give a compound **25**. The thiol moiety is nitrosated with a suitable mild nitrosylating agent such as sodium nitrite in aqueous hydrochloric acid or t-butyl nitrite to afford compound **26**. Compound **26** is then converted to the complex of formula **VA** by reacting with a manganese II compound under aerobic conditions.

Compounds of formula VI, wherein d, d', c, E and L are as defined above, can be prepared according the reaction scheme depicted in Figure 6, in which a desferroxamine derived manganese chelate is representative of the transition metal chelates defined above. The amine group of the compound of formula **27** is reacted with a compound of formula **23**, wherein T, c and P^2 are defined as above, to afford a compound of formula **28**. For example, a thiol containing acid with the thiol moiety protected as the S-(N-methoxymethylcarbamate), is converted to the acid chloride **23a** with oxalyl chloride and a catalytic amount of DMF and **23a** is reacted with **27** in an aprotic solvent such as DMF or THF to afford the amide of formula **28a**. Alternatively, the thiol containing acid with the thiol moiety protected as the S(N-methoxymethyl-carbamate), is converted to the acyl azide by treatment with diphenylphosphoryl azide and this intermediate is heated to catalyze the Curtius rearrangement to the corresponding isocyanate **23b**. Treatment of **23b** with a compound of formula **27** affords a urea of formula **28b**. Alternatively, the thiol containing acid with the thiol moiety protected as the S-(N-methoxymethyl-carbamate), is reduced to the alcohol with borane. Treatment of the alcohol with one equivalent of phosgene affords the chloroformate **23c** which is then reacted with a compound of formula **27** to produce the carbamate of

formula **28c**. The thiol protecting group is cleaved (aqueous base is used to remove S-(N-methoxymethyl-carbamate) groups) and the thiol moiety is nitrosated with a suitable mild nitrosylating agent such as sodium nitrite in aqueous hydrochloric acid or t-butyl nitrite in chloroform to afford the compound of formula **29**. The compound of formula **29** is then converted to the complex of formula VIA by reacting with a manganese II compound under aerobic conditions.

Compounds of formula VII, wherein M, R^7, X, D, and L are as defined above, can be prepared according the reaction scheme depicted in Figure 7, in which a manganese salen complex is representative of the transition metal chelates defined above. A thiol containing α-amino acid of formula **9** with the nitrogen and sulfur moieties protected, wherein a, P^1, and P^2 are as defined above, is activated at the carboxylate with a suitable activating agent such that, in the presence of ammonia, it forms the amide of formula **30**. Preferred protecting groups for the α-amino acid are a t-Boc for the amine and a benzyl group for the thiol. For example, one method of activation of the carboxylate group is formation of the mixed anhydride by reaction of the acid with isobutylchloroformate in the presence of a base such as N-methylmopholine and subsequent addition of ammonia affords the compound of formula **30**. The amide of formula **30** is converted to the diamine of formula **31** by removing the nitrogen protecting group and then reducing the amide functionality. For example, cleavage of a t-butoxycarbamate groups is accomplished with a strong acid such as trifluoroacetic acid in methylene chloride or 4N hydrochloric acid in dioxane. The amide is then reduced with a reducing agent such as lithium aluminum hydride or borane in an aprotic solvent such as ether or THF. Treatment of the compound of formula **31** with a compound of formula **32** in a protic solvent such as ethanol affords a compound of formula

33. The compound of formula 33 is converted to the compound of formula 34 by removing the sulfur protecting group and nitrosylating the free thiol. For example, thiobenzyl groups can be cleaved with sodium in liquid ammonia and nitrosation of the thiol group is accomplished using a suitable mild nitrosating agent such as sodium nitrite in aqueous hydrochloric acid or dinitrogen tetroxide in chloroform. The compound of formula 34 is then converted into the complex of formula **VIIA** by reacting with a manganese II compound under aerobic conditions.

Contemplated equivalents of the general formulas set forth above for the compounds and derivatives as well as the intermediates are compounds otherwise corresponding thereto and having the same general properties such as tautomers of the compounds and such as wherein one or more of the various R groups are simple variations of the substituents as defined therein, e.g., wherein R is a higher alkyl group than that indicated, or where the tosyl groups are other nitrogen or oxygen protecting groups or wherein the tosyl is a halide. Anions having a charge other than 1, *e.g.*, carbonate, phosphate, and hydrogen phosphate, can be used instead of anions having a charge of 1, so long as they do not adversely affect the overall activity of the complex. However, using anions having a charge other than 1 will result in a slight modification of the general formula for the complex set forth above. In addition, where a substituent is designated as, or can be, a hydrogen, the exact chemical nature of a substituent which is other than hydrogen at that position, *e.g.*, a hydrocarbyl radical or a halogen, hydroxy, amino and the like functional group, is not critical so long as it does not adversely affect the overall activity and/or synthesis procedure. Further, it is contemplated that manganese(III) complexes will be equivalent to the subject manganese(II) complexes.

The chemical reactions described above are generally disclosed in terms of their broadest application to the preparation of the compounds of this invention. Occasionally, the reactions may not be applicable as described to each compound included within the disclosed scope. The compounds for which this occurs will be readily recognized by those skilled in the art. In all such cases, either the reactions can be successfully performed by conventional modifications known to those skilled in the art, *e.g.*, by appropriate protection of interfering groups, by changing to alternative conventional reagents, by routine modification of reaction conditions, and the like, or other reactions disclosed herein or otherwise conventional, will be applicable to the preparation of the corresponding compounds of this invention. In all preparative methods, all starting materials are known or readily preparable from known starting materials.

Activity of the compounds or complexes of the present invention which catalyze the dismutation of superoxide can be demonstrated using the stopped-flow kinetic analysis technique as described in Riley *et al.*, *Anal. Biochem.*, *196*: 344-349 (1991), which is incorporated by reference herein. Stopped-flow kinetic analysis is an accurate and direct method for quantitatively monitoring the decay rates of superoxide in water. The stopped-flow kinetic analysis is suitable for screening compounds for SOD activity and activity of the compounds or complexes of the present invention, as shown by stopped-flow analysis, correlate to treating the disease states and disorders identified herein.

The compounds or complexes of the present invention are novel and can be utilized to treat numerous inflammatory disease states and disorders. For example, reperfusion injury to an ischemic organ, *e.g.*, reperfusion injury to the

ischemic myocardium, myocardial infarction, inflammatory bowel disease, rheumatoid arthritis, osteoarthritis, hypertension, psoriasis, organ transplant rejections, organ preservation, impotence, radiation-induced injury, asthma, atherosclerosis, thrombosis, platelet aggregation, metastasis, influenza, stroke, burns, trauma, acute pancreatitis, pyelonephritis, hepatitis, autoimmune diseases, insulin-dependent diabetes mellitus, disseminated intravascular coagulation, fatty embolism, adult and infantile respiratory distress, carcinogenesis and hemorrhages in neonates.

The invention also relates to pharmaceutical compositions containing the nitroso-SOD or nitrosylated compounds with SOD-like activity as well as nitro-SOD or nitrated compounds with SOD-like activity, together with a pharmaceutically acceptable carrier.

While the compounds of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination or concurrently with one or more compounds which are known to be effective against the specific disease state that one is targeting for treatment. Particularly contemplated in this respect are nitric oxide adducts, such as are discussed below.

Further, the invention provides that the superoxide oxidants or reductants which have not been linked to an NO or NO_2 group can be administered in combination or concurrently with nitric oxide or nitric oxide adducts. Thus, this aspect pharmaceutical composition comprising a superoxide oxidant or reductant and a nitric oxide adduct in a pharmaceutically acceptable carrier.

In this aspect, the superoxide oxidant or reductant can be a superoxide dismutase, for example a manganese-containing superoxide dismutase, an iron-containing superoxide dismutase or a copper and zinc-containing superoxide dismutase.

Alternatively, the superoxide oxidant or reductant can be a superoxide dismutase mimetic. These can include, for example, those having the formula

$$\begin{array}{ccccccc} & |\,\alpha & & & & |\,\alpha^1 & \\ - & C & - & N & - & C & - \\ & | & & \downarrow & & | & \\ & & & OR & & & \end{array}$$

wherein R is an unpaired electron, a cation such as a physiologically acceptable metal ion, hydrogen or a protective group; and the α and α^1 carbons are substituted such that when R is an unpaired electron the compound is a stable nitroxide.

Other examples of applicable superoxide dismutase mimetics include: metal independent nitroxide compounds; spiro[cyclohexane-1,2'-(4',4'-dimethyloxazolidine-3'-oxyl)], 2-ethyl-2,4,4-trimethyl oxazolidine-3-oxyl; 3-carboxy-2,2,5,5-tetramethylproxyl; manganese desferroxamine complexes; macrocyclic ligands such as 1,4,7,10,13-pentaaxacyclopentadecane; salen-manganese complexes; monomeric(benzoato) manganese (II) SOD mimetic complexes; Cu(II) desferritiocin complexes; diSchiff base coordinated copper complexes; and metal complexes of a pyrimine.

Compounds contemplated for use in combination or concurrently with the nitrosylated and nitrated superoxide oxidants and reductants of the invention are nitric oxide and compounds referred to herein as "nitric oxide adducts" that release nitric oxide or otherwise directly or indirectly deliver or transfer nitric oxide to a site of its activity,

such as on a cell membrane, *in vivo*. As used here, the term "nitric oxide" encompasses uncharged nitric oxide(NO●) and charged nitric oxide species, particularly including nitrosonium ion(NO^+) and nitroxyl ion(NO^-). The reactive form of nitric oxide can be provided by gaseous nitric oxide. The nitric oxide releasing, delivering or transferring compounds, having the structure X-NO wherein X is a nitric oxide releasing, delivering or transferring moiety, include any and all such compounds which provide nitric oxide to its intended site of action in a form active for their intended purpose. As used here, the term "nitric oxide adducts" encompasses any of such nitric oxide releasing, delivering or transferring compounds, including, for example, S-nitrosothiols, S-nitroso amino acids, S-nitroso-polypeptides, nitrites and nitrosoamines. It is contemplated that any or all of these "nitric oxide adducts" can be mono- or poly- nitrosylated at a variety of naturally susceptible or artificially provided binding sites for nitric oxide.

One group of such nitric oxide adducts is the S-nitrosothiols, which are compounds that include at least one -S-NO group. Such compounds include S-nitroso-polypeptides (the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function, and derivatives thereof); S-nitrosylated amino acids(including natural and synthetic amino acids and their stereoisomers and racemic mixtures and derivatives thereof); S-nitrosated sugars, S-nitrosated-modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200, nucleotides); and an S-nitrosated hydrocarbon where the hydrocarbon can be a branched or unbranched, and saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon; S-nitroso hydrocarbons having one or more substituent groups in addition to the S-nitroso group; and heterocyclic compounds.

S-nitrosothiols and the methods for preparing them are described in U.S. Patent Application No. 07/943,834, filed September 14, 1992, Oae *et al.*, *Org. Prep. Proc. Int.*, *15(3)*:165-198, 1983; Loscalzo *et al.*, *J. Pharmacol. Exp. Ther.*, *249(3)*:726729, 1989, and Kowaluk *et al.*, *J. Pharmacol. Exp. Ther.*, *256*:1256-1264, 1990, all of which are incorporated in their entirety by reference.

One particularly preferred embodiment of this aspect relates to S-nitroso amino acids where the nitroso group is linked to a sulfur group of a sulfur-containing amino acid or derivative thereof. For example, such compounds include the following: S-nitroso-N-acetylcysteine, S-nitroso-captopril, S-nitroso-homocysteine, S-nitroso-cysteine and S-nitroso-glutathione.

Suitable S-nitrosylated proteins include thiol-containing proteins (where the NO group is attached to one or more sulfur group on an amino acid or amino acid derivative thereof) from various functional classes including enzymes, such as tissue-type plasminogen activator (TPA) and cathepsin B; transport proteins, such as lipoproteins, heme proteins such as hemoglobin and serum albumin; and biologically protective proteins, such as the immunoglobulins and the cytokines. Such nitrosylated proteins are described in PCT Publ. Applic. No. WO 93/09806, published May 27, 1993. Examples include polynitrosylated albumin where multiple thiol or other nucleophilic centers in the protein are modified.

Further examples of suitable S-nitrosothiols include those having the structures:

(i) $CH_3(CH_2)_xSNO$

wherein x equals 2 to 20;

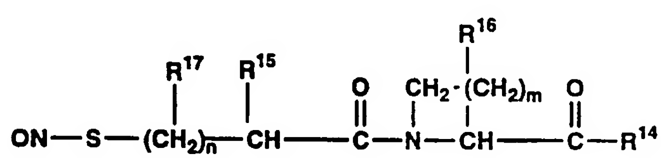
(ii) $HS(CH_2)_xSNO$

wherein x equals 2 to 20; and

(iii) $ONS(CH_2)_xY$

wherein x equals 2 to 20 and Y is selected from the group consisting of halo, alkoxy, cyano, carboxamido, cycloalkyl, arylalkoxy, lower alkylsulfinyl, arylthio, alkylamino, dialkylamino, hydroxy, carbamoyl, N-alkylcarbamoyl, N,N-dialkylcarbamoyl, amino, hydroxyl, carboxyl, hydrogen, nitro and aryl.

Other suitable S-nitrosothiols that are S-nitroso-angiotensin converting enzyme inhibitors (hereinafter referred to as S-nitroso-ACE inhibitors) are described in Loscalzo, U.S. Patent No. 5,002,964 (1991) and Loscalzo *et al.*, U.S. Patent No. 5,025,001 (1991) both of which are incorporated in their entirety by reference. Examples of such S-nitroso-ACE inhibitors include compounds having the following structure:



wherein

R^{14} is hydroxy, NH_2, NHR^{24}, $NR^{24}R^{25}$, or lower alkoxy, wherein R^{24} and R^{25} are alkyl, or aryl, or arylalkyl;

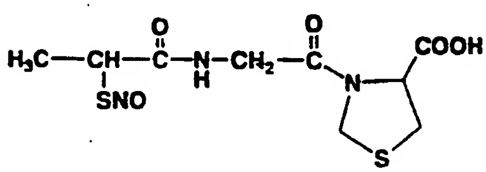
R^{15} is hydrogen, alkyl, arylalkyl, amino, guanidino, NHR^{26}, $N^1R^{26}R^{27}$, wherein R^{26} and R^{27} are methyl or alkanoyl;

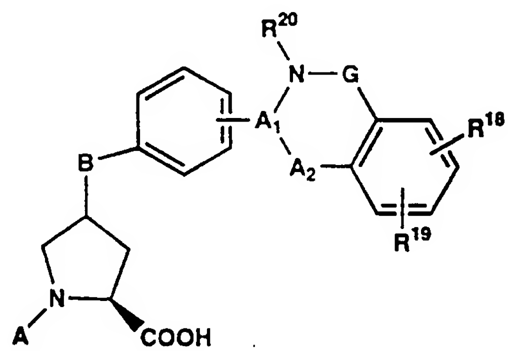
R^6 is hydrogen, hydroxy, C_1-C_4 alkoxy, phenoxy, or lower alkyl;

R^{17} is hydrogen, alkyl or arylalkyl;
m is 1 to 3; and
n is 0 to 4.

Other suitable S-nitroso-ACE inhibitors include N-acetyl-S-nitroso-D-cysteinyl-L-proline, N-acetyl-S-nitroso-D,L-cysteinyl-L-proline, 1-[4-amino-2-(S-nitroso)mercaptomethyl butanoyl]-L-proline, 1-[2-hexanoyl]-L-proline, 1-[5--guanidino-2-(S-nitroso)mercaptomethyl-pentanoyl]-L-proline, 1-[5-amino-2-(S-nitroso) mercaptomethyl-pentanoyl]-4-hydroxy-L-proline, 1-[5-guanidino-2-(S-nitroso)mercaptomethyl-pentanoyl]-4-hydroxy-L-proline, 1-[2-aminomethyl-3(S-nitroso)-mercaptomethyl-pentanoyl-L-proline, andS-nitroso-L-cysteinyl-L-proline.

Additional suitable S-nitroso-ACE inhibitors include those having the following structures:





wherein

B is oxygen or sulfur;

$-A_1,-A_2-$ is CH-NH or -C=N-;

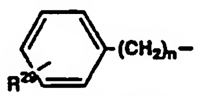
A is $\mathrm{ON\text{-}\overset{R^{28}}{\overset{|}{S}}\text{-}CH_2\text{-}CH\text{-}\overset{O}{\overset{\|}{C}}}$;

R^{28} is selected from hydrogen, alkyl, arylalkyl, and salt forming ion;

R^{18} and R^{19} are independently selected from hydrogen, halogen, alkyl, alkoxy, halo substituted lower alkyl, nitro, and SO_2NH_2;

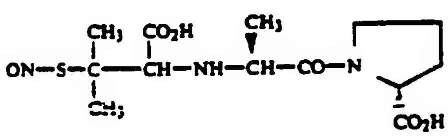
G is $-\overset{O}{\overset{\|}{C}}-$ or $-\overset{O\ \ O}{\overset{\diagdown\ \diagup}{S}}-$

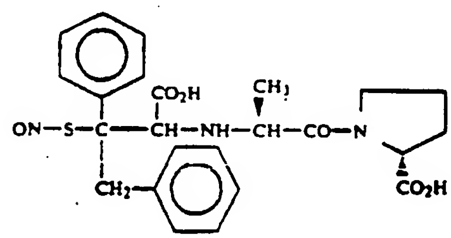
R^{20} is hydrogen, lower alkyl, halo substituted lower alkyl, hydroxy substituted lower alkyl, $-(CH_2)_q-N$ (lower alkyl)$_2$ or $-(CH_2)_q-NH_2$ and q is one, two, three or four; and

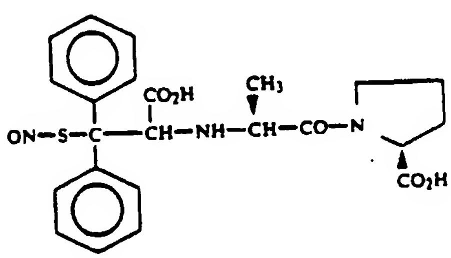


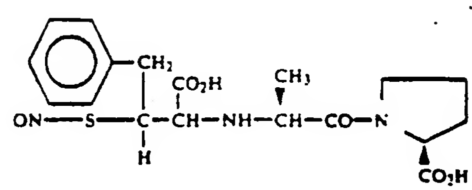
wherein R^{29} is hydrogen, lower alkyl, alkoxy, halogen or hydroxy and h is as defined above.

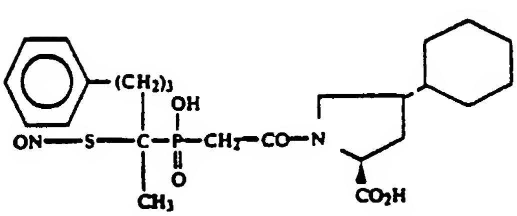
Additional suitable compounds include those having the following structures:

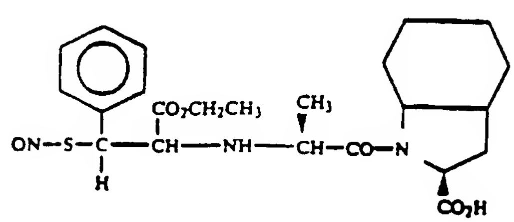


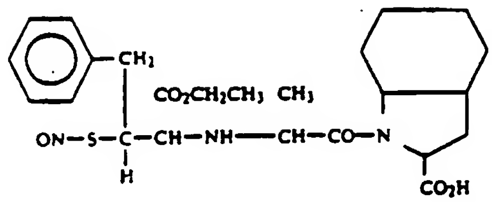


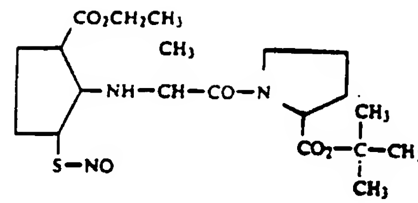












The S-nitroso-ACE inhibitors can be prepared by various methods of synthesis. In general, the thiol precursor is prepared first, then converted to the S-nitrosothiol derivative by nitrosation of the thiol group with $NaNO_2$ under acidic conditions (pH = 1 to 5) which yields the S-nitroso derivative. Acids which may be used for this purpose include aqueous sulfuric, acetic and hydrochloric acids. Thiol precursors are prepared as described in the following: U.S. Pat. Nos. 4,046,889 (1977); 4,052,511; 4,053,651; 4,113,751, 4,154,840, 4129,571 (1978), and 4,154,960 (1979) to Ondetti *et al.*; U.S. Pat. No. 4,626,545 (1986) to Taub; and U.S. Pat. Nos. 4,692,458 (1987) and 4,692,459 (1987) to Ryan *et al.*, Quadro, U.S. Pat. No. 4,447,419 (1984); Haugwitz *et al.*; U.S. Pat. No. 4,681,886 (1987), Bush *et al.*, U.S. Pat. No. 4,568,675 (1986), Bennion *et al.*, U.S. Pat. No. 4,748,160 (1988), Portlock, U.S. Pat. No. 4,461,896 (1984), Hoefle *et al.*, European Patent Application Publication No. 0 088 341 (1983), Huange *et al.*, U.S. Pat. No. 4,585,758 (1986), European Patent application Publication No. 0 237 239, European Patent application Publication No. 0 174 162, published in 1986, European Patent application Publication No. 0 257 485, published in 1988, all of which are incorporated by reference herein.

Another group of such NO adducts are compounds that include at least one -O-NO group. Such compounds include O-

nitroso-polypeptides (the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function, and derivatives thereof); O-nitrosylated amino acids (including natural and synthetic amino acids and their stereoisomers and racemic mixtures and derivatives thereof); O-nitrosated sugars; O-nitrosated-modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200, nucleotides); and an O-nitrosated hydrocarbon where the hydrocarbon can be a branched or unbranched, saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon; O-nitroso hydrocarbons having one or more substituent groups in addition to the O-nitroso group; and heterocyclic compounds.

Another group of such NO adducts is the nitrites which have an -O-NO group wherein R is a protein, polypeptide, amino acid, branched or unbranched and saturated or unsaturated alkyl, aryl or a heterocyclic. A preferred example is the nitosylated form of isosorbide. Compounds in this group form S-nitrosothiol intermediates *in vivo* in the recipient human or other animal to be treated and can therefore include any structurally analogous precursor R-O-NO of the S-nitrosothiols described above.

Another group of such NO adducts is the N-nitrosoamines, which are compounds that include at least one -N-NO group. Such compounds include N-nitroso-polypeptides (the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function, and derivatives thereof); N-nitrosylated amino acids (including natural and synthetic amino acids and their stereoisomers and racemic mixtures); N-nitrosated sugars; N-nitrosated-modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200, nucleotides); and an N-nitrosated hydrocarbon where the hydrocarbon can be a

branched or unbranched, and saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon; N-nitroso hydrocarbons having one or more substituent groups in addition to the N-nitroso group; and heterocyclic compounds.

Another group of such NO adducts is the C-nitroso compounds that include at least one -C-NO group. Such compounds include C-nitroso-polypeptides (the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function, and derivatives thereof); C-nitrosylated amino acids (including natural and synthetic amino acids and their stereoisomers and racemic mixtures); C-nitrosated sugars; C-nitrosated-modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200, nucleotides); and a C-nitrosated hydrocarbon where the hydrocarbon can be a branched or unbranched, and saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon; C-nitroso hydrocarbons having one or more substituent groups in addition to the C-nitroso group; and heterocyclic compounds.

Another group of such NO adducts is the nitrates which have at least one -O-NO_2 group. Such compounds include polypeptides (the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function, and derivatives thereof); amino acids (including natural and synthetic amino acids and their stereoisomers and racemic mixtures and derivatives thereof); sugars; modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200, nucleotides); and a hydrocarbon where the hydrocarbon can be a branched or unbranched, and saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon; hydrocarbons having one or more substituent groups; and heterocyclic compounds. A preferred example is nitroglycerin.

Another group of such NO adducts is the nitroso-metal compounds which have the structure $(R)_n$-A-M-$(NO)_x$. R includes polypeptides(the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function, and derivatives thereof); amino acids (including natural and synthetic amino acids and their stereoisomers and racemic mixtures and derivatives thereof); sugars; modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200, nucleotides); and a hydrocarbon where the hydrocarbon can be a branched or unbranched, and saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon; hydrocarbons having one or more substituent groups in addition to the A-nitroso group; and heterocyclic compounds. A is S, O, or N, n and x are each integers independently selected from 1, 2 and 3, and M is a metal, preferably a transition metal. Preferred metals include iron, copper, manganese, cobalt, selenium and luthidium. Also contemplated are N-nitrosylated metal centers such as nitroprusside.

Another group of such NO adducts is the N-oxo-N-nitrosoamines which have an R-N(O^-M^+)-NO group or an R-NO-NO-group. R includes polypeptides (the term "polypeptide" includes proteins and also polyamino acids that do not possess an ascertained biological function, and derivatives thereof); amino acids(including natural and synthetic amino acids and their stereoisomers and racemic mixtures and derivatives thereof); sugars; modified and unmodified oligonucleotides (preferably of at least 5, and more particularly 5-200, nucleotides); and a hydrocarbon where the hydrocarbon can be a branched or unbranched, and saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon; hydrocarbons having one or more substituent groups; and heterocyclic compounds. R is

preferably a nucleophilic (basic) moiety. M+ is a metal cation, such as, for example, a Group I metal cation.

Another group of such NO adducts is the thionitrates which have the structure R-$(S)_x$-NO wherein x is an integer of at least 2. R is as described above for the S-nitrosothiols. Preferred are the dithiols wherein x is 2. Particularly preferred are those compounds where R is a polypeptide or hydrocarbon and a pair or pairs of thiols are sufficiently structurally proximate, *i.e.* vicinal, that the pair of thiols will be reduced to a disulfide. Those compounds which form disulfide species release nitroxyl ion(NO^-) and uncharged nitric oxide(NO●). Those compounds where the thiol groups are not sufficiently close to form disulfide bridges generally only provide nitric oxide as the NO^- form not as the uncharged NO● form.

As noted above, the invention further provides various embodiments of pharmaceutical preparations. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

The compounds of the present invention may be administered orally, parenterally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants and vehicles as desired. Topical administration may also involve the use of transdermal administration such as transdermal patches or iontophoresis devices. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection and infusion techniques.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, can be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1, 3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed an a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. in addition, fatty acids such as oleic acid find use in the preparation of injectables.

Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter and polyethylene glycols which are solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, granules and gels. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose lactose or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

The dosage regimen for treating a disease condition with the compounds and/or compositions of this invention is selected in accordance with a variety of factors, including the type, age, weight, sex, diet and medical condition of the patient, the severity of the disease, the route of administration, pharmacological considerations such as the activity, efficacy, pharmacokinetic and toxicology profiles of the particular compound employed, whether a drug delivery system is utilized and whether the compound is administered as part of a drug combination. Thus, the dosage regimen actually employed may vary widely and therefore may deviate from the preferred dosage regimen set forth herein.

Total daily dose administered to a host in single or divided doses may be in amounts, for example, from about 1 to about 100 mg/kg body weight daily and more usually about 3 to 30 mg/kg. Dosage unit compositions may contain such amounts of submultiples thereof to make up the daily dose.

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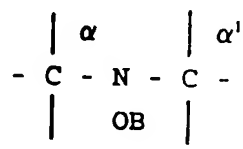
What Is Claimed Is:

1. A compound comprising a superoxide oxidant or reductant to which is directly or indirectly linked an NO or NO_2 group.

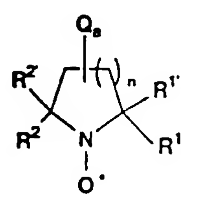
2. The compound of claim 1 which has the formula



wherein B is an unpaired electron, a physiologically acceptable metal ion, hydrogen or a protective group; and at least one of the α and α' carbons is directly or indirectly linked to an NO or NO_2 group.

3. The compound of claim 2 selected from the group consisting of:

(I) a compound having the formula



I

wherein

n is an integer from 0 to 4;

R^1, $R^{1'}$, R^2, and $R^{2'}$ are each independently selected from the group consisting of:

(i) $-(CH_2)_a$-X-D, in which a is an integer from 1 to 4 and X is S, O, C, N(CO)R° or NR°, in which R° is H,

(i) $-(CH_2)_a$-X-D, in which a is an integer from 1 to 4 and X is S, O, C, N(CO)R^O or NR^O, in which R^O is H,

alkyl, cycloalkyl, aryl, heteroaryl or a heterocyclic ring system; and D is NO or NO_2;

(ii) alkyl;

(iii) $-(CH_2)_aR^3$, in which a is as defined above and R^3 is cycloalkyl, aryl, heteroaryl or a heterocyclic ring system;

(iv) $-(CH_2)_aOR^4$, in which a is as defined above and R^4 is cycloalkyl, alkyl, heteroaryl or a heterocyclic ring system;

(v) $-(CH_2)_aNHR^4$, in which a and R^4 are as defined above;

or in which, when taken together, one or both of R^1 and $R^{1\prime}$ or R^2 and $R^{2\prime}$ are selected from the group consisting of cycloalkyl, norbornyl and cholestane; and

Q_a, in which a is as defined above, is located on the nitrogen-containing ring at one or more positions not substituted by R^1, $R^{1\prime}$, R^2, or $R^{2\prime}$ and is selected from the group consisting of:

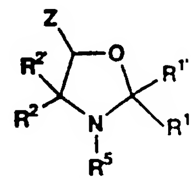
(i) $-(CH_2)_b$-X-D, in which b is an integer from 0 to 4, and X and D are as defined above;

(ii) -C(O)X-$(CH_2)_c$-X-D, in which c is an integer from 2 to 6 and X and D are as defined above;

(iii) -X-[C(O)C($(CH_2)_a$-X-D)-NH]$_a$p' wherein p' is a nitrogen protecting group and X, D, and a are defined as above; and

(iv) H, with the proviso that at least one of R^1, $R^{1\prime}$, R^2 or $R^{2\prime}$ consists of $-(CH_2)_a$-X-D; in which a, X and D are as defined above.

(II) a compound having the formula



II

wherein

R^1, $R^{1'}$, R_2 and $R^{2'}$ are each independently selected from the group consisting of:

(i) $-(CH_2)_a$-X-D, in which a is an integer from 1 to 4 and X is S, O, C or NR^O, in which R^O is H, alkyl, cycloalkyl, heteroaryl or a heterocyclic ring system; and D is NO or NO_2;

(ii) alkyl;

(iii) $-(CH_2)_aR^3$, in which a is as defined above and R^3 is cycloalkyl, alkyl, heteroaryl or a heterocyclic ring system;

(iv) $-(CH_2)_aOR^4$, in which a is as defined above and R^4 is cycloalkyl, alkyl, heteroaryl or a heterocyclic ring system;

(v) $-(CH_2)_aNHR^4$, in which a and R^4 are as defined above; or in which, when taken together, one or both of R^1 and $R^{1'}$ or R^2 and $R^{2'}$ are selected from the group consisting of cycloalkyl, norbornyl and cholestane;

Z is selected from the group consisting of:

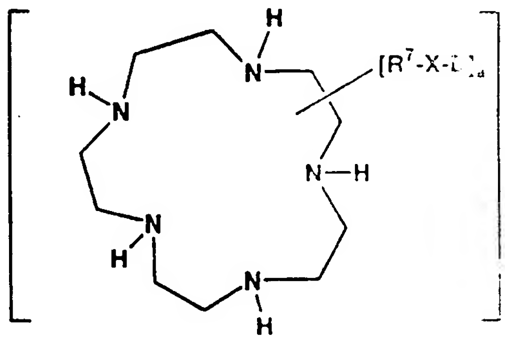
(i) $-(CH_2)_a$-X-D, in which a is an integer from 1 to 4; X is S, O, C, $N(CO)R^O$ or NR^O, in which R^O is H, alkyl, cycloalkyl, heteroaryl or a heterocyclic ring system; and D is NO or NO_2

(ii) $-C(O)X-(CH_2)_c$-X-D, in which c is an integer from 2 to 6 and X and D are as defined above; and

(iii) H, with the proviso that at least one of R^1, $R^{1'}$, R^2, or $R^{2'}$ consists of $-(CH_2)_a-X-D$; in which a, X and D are defined as above; and

R^5 is selected from the group consisting of O• and OH;

(III) a compound having the formula

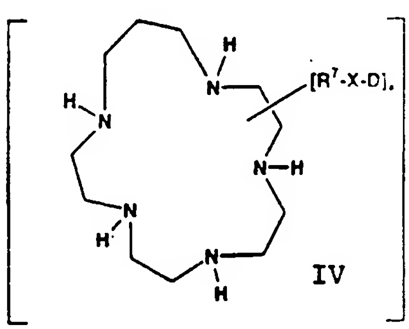


M-L_n

III

wherein M is iron, copper or manganese; L is at least one suitable organic or inorganic ligand or organic or inorganic charge-neutralizing anion which is derived from any monodentate or polydentate coordinating ligand or ligand system or the corresponding anion thereof; X is S, O, C, N(CO)R^O or NR^O, in which R^O is H, alkyl, cycloalkyl, heteroaryl or a heterocyclic ring system; and a is an integer from 1 to 4;

(IV) a compound having the formula

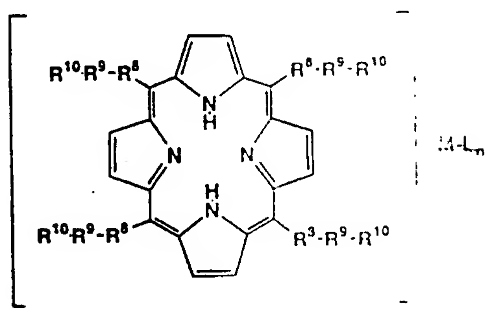


M-L_n

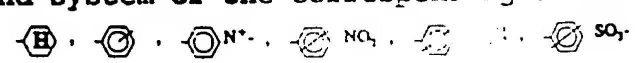
IV

wherein M is iron, copper or manganese; L is at least one suitable organic or inorganic ligand or organic or inorganic charge-neutralizing anion which is derived from any monodentate or polydentate coordinating ligand or ligand system or the corresponding anion thereof; X is S, O, C, N(CO)R^{O} or NR^{O}, in which R^{O} is H, alkyl, cycloalkyl, heteroaryl or a heterocyclic ring system; and a is an integer from 1 to 4;

(V) compound having the formula

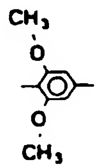


V

or a pharmaceutically acceptable salt thereof wherein:
M is iron, copper or manganese;
L is at least one suitable organic or inorganic ligand or organic or inorganic charge-neutralizing anion which is derived from any monodentate or polydentate coordinating ligand or ligand system or the corresponding anion thereof;
R^{8} is a bond, 

  wherein U is a halogen and V is an alkyl group and wherein  indicates bonding to R^{9} at any position and  indicates bonding to R^{9} and the substituent at any position; and

R^9 is a bond, $-(CY'_2)_n-$, $-(CY'_2-CY'=CY')_n-$, $-(CY'_2-CY'_2-CH=CH)_n-$ or $-(CY'_2-C)_n-$, wherein Y' is hydrogen or an alkyl group and wherein n is 1 to 8; or



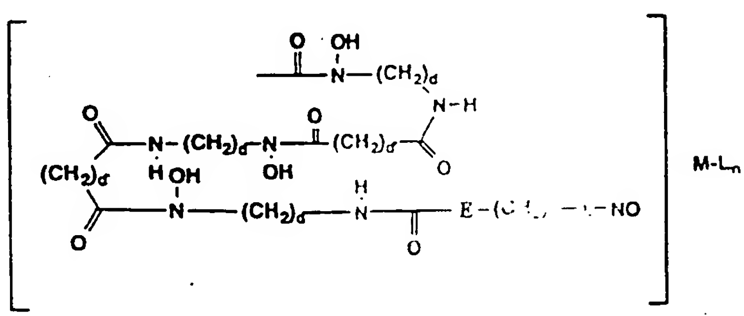
; and

R^{10} is -X-D, $-CH_2-CH_2-C-\underset{CH_3}{\overset{CH_3}{X}}-D$, $-(CH_2)_{1-3}-X-D$, -NH-D or NO^--D,

in which X is S, O, C, $N(CO)R^0$ or NR^0 in which R^0 is H, O, alkyl, cycloalkyl, heteroaryl or a heterocyclic ring system; and

D is NO or NO_2.

(VI) a compound having the formula



VI

wherein

M is iron, copper or manganese;

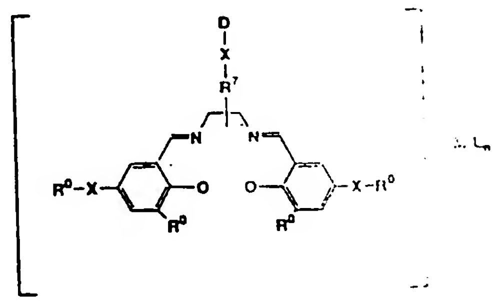
X is S, O, C, $N(CO)R^0$ or NR^0, in which R^0 is H, alkyl, cycloalkyl, heteroaryl or a heterocyclic ring system;

E is (i) a covalent bond
(ii) X wherein X is defined as above.

L is a suitable organic or inorganic ligand or organic or inorganic charge-neutralizing anion which is derived from any monodentate or polydentate coordinating ligand or ligand system or the corresponding anion thereof; and
d and d′ are each independently an integer from 1 to 6;

and

(VII) a compound having the formula



VII

wherein

M is iron, copper or manganese;

X is S, O, C, N(CO)R^O or NR^0, in which R^O is H, alkyl, cycloalkyl, heteroaryl or a heterocyclic ring system;

L is a suitable organic or inorganic ligand or organic or inorganic charge-neutralizing anion which is derived from any monodentate or polydentate coordinating ligand or ligand system or the corresponding anion thereof; and

a is an integer from 1 to 4.

4. The compound of claim 1 wherein the superoxide oxidant or reductant is a metal complex of a transition metal and a macrocyclic ligand.

5. The compound of claim 4 having the formula:

$$R^6[R^7\text{-}X\text{-}D]_a$$

wherein R^6 is a complex of a transition metal and a macrocyclic ligand that dismutes superoxide under physiological conditions; R^7 is a bond or a linking group; X is S, O, C or NR^o, in which R^o is H, alkyl, cycloalkyl, heteroaryl or a heterocyclic ring system; D is NO or NO_2; and a is an integer from 1 to 4.

6. The compound of claim 5 wherein the macrocyclic ligand is a porphyrin.

7. The compound of claim 6 wherein the porphyrin is tetrakis (4-benzoic acid) porphyrin.

8. The compound of claim 1 wherein the superoxide oxidant or reductant is a superoxide dismutase.

9. The compound of claim 8 wherein the superoxide dismutase is selected from the group consisting of a manganese-containing superoxide dismutase, an iron-containing superoxide dismutase and a copper and zinc-containing superoxide dismutase.

10. A compound comprising a superoxide oxidant or reductant to which is directly or indirectly linked (i) an NO or NO_2 group and (ii) a specific binding ligand.

11. The compound of claim 10 wherein the specific binding ligand is specifically bindable with a cell surface or extra- cellular matrix specific binding partner.

12. The compound of claim 10 wherein the superoxide oxidant or reductant is linked to the N_2O_2 group through the specific binding ligand.

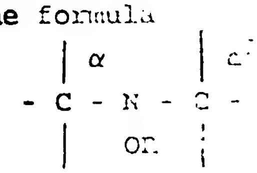
13. A pharmaceutical composition comprising the compound of claim 1 in a pharmaceutically acceptable carrier.

14. A pharmaceutical composition comprising the compound of claim 10 in a pharmaceutically acceptable carrier.

15. A pharmaceutical composition comprising a superoxide oxidant or reductant and a nitric oxide adduct in a pharmaceutically acceptable carrier.

16. The composition of claim 15 wherein the superoxide oxidant or reductant is a superoxide dismutase mimetic.

17. The composition of claim 16 wherein the superoxide dismutase mimetic has the formula



wherein R is an unpaired electron, [illegible] ion such as a physiologically acceptable metal ion, hydrogen or a protective group; and the α and α^1 [illegible] are substituted such that when R is an unpaired electron the compound is a stable nitroxide.

18. The composition of claim 17 [illegible] the superoxide dismutase mimetic is selected from the [illegible] consisting of a metal independent nitroxide compound; [illegible] cyclohexane-1,2'-(4',4'-dimethyloxazolidine-3'-oxyl)], [illegible]-2,4,4-trimethyl oxazolidine-3-oxyl; 3-carboxy-2,2,[illegible]tramethylproxyl;

manganese desferroxamine complexes; ma ... ic ligands such as 1,4,7,10,13-pentaaxacyclopentadec... salen-manganese complexes; monomeric(benzoato) mangan... I) SOD mimetic complexes; Cu(II) desferritiocin comp... diSchiff base coordinated copper complexes; and m... omplexes of a pyrimine.

19. The composition of clai... 15 ... the superoxide oxidant or reductant is a superoxid... e.

20. The composition of claim 19 ... the superoxide dismutase is selected from the gro... nsisting of a manganese-containing superoxide dismut... iron-containing superoxide dismutase and a copper ... zinc-containing superoxide dismutase.

21. A method of protecting c... rom superoxide radical-induced toxicity compri...ing ... ing said cells with a protective amount of the compo... f claim 13.

22. A method of protecting ... rom superoxide radical-induced toxicity comprising ... ing said cells with a protective amount of the compo... f claim 14.

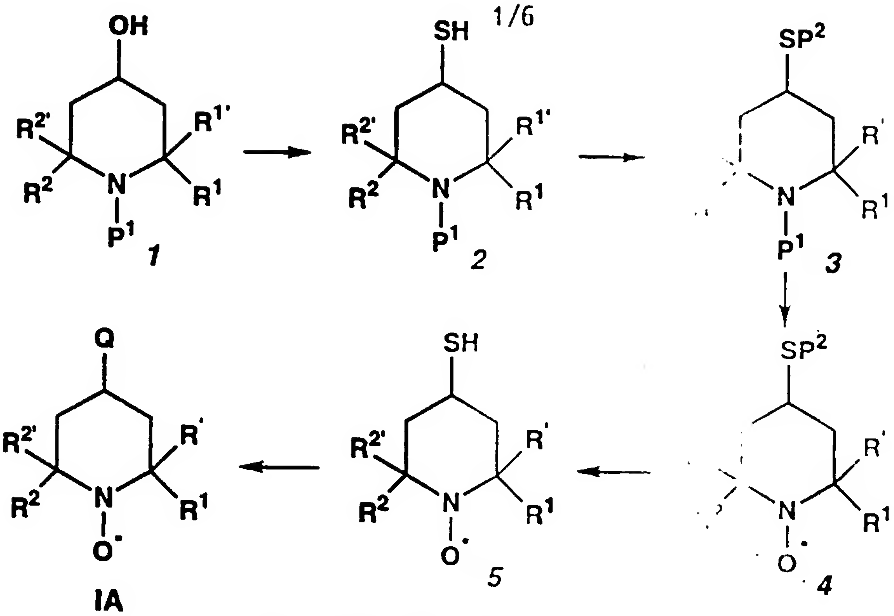
23. A method of protecting ... rom superoxide radical-induced toxicity comprising ... ing said cells with a protective amount of the comp... f claim 15.

24. A method for treating an in... ry condition in a patient in need of such treatment co... g administering to said patient a therapeutic ... unt ... composition of claim 13.

25. A method for treating an inf... ry condition in a patient in need of such treatment c... g administering

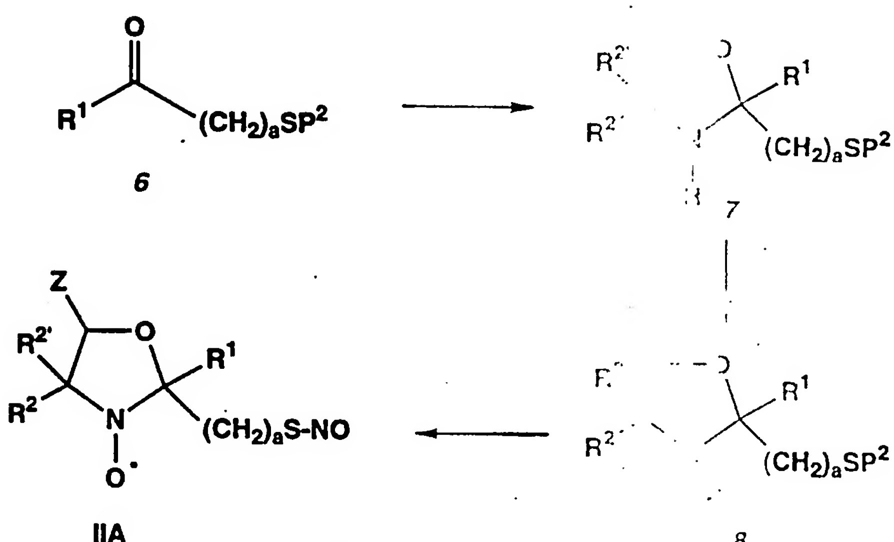
to said patient a therapeutic amount of the composition of claim 14.

26. A method for treating an inflammatory condition in a patient in need of such treatment comprising administering to said patient a therapeutic amount of the composition of claim 15.



$R^1, R^{1'}, R^2, R^{2'} = CH_3$ Q = SNO

Figure 1



$R^2, R^{2'} = CH_3$ Z = !

Figure 2

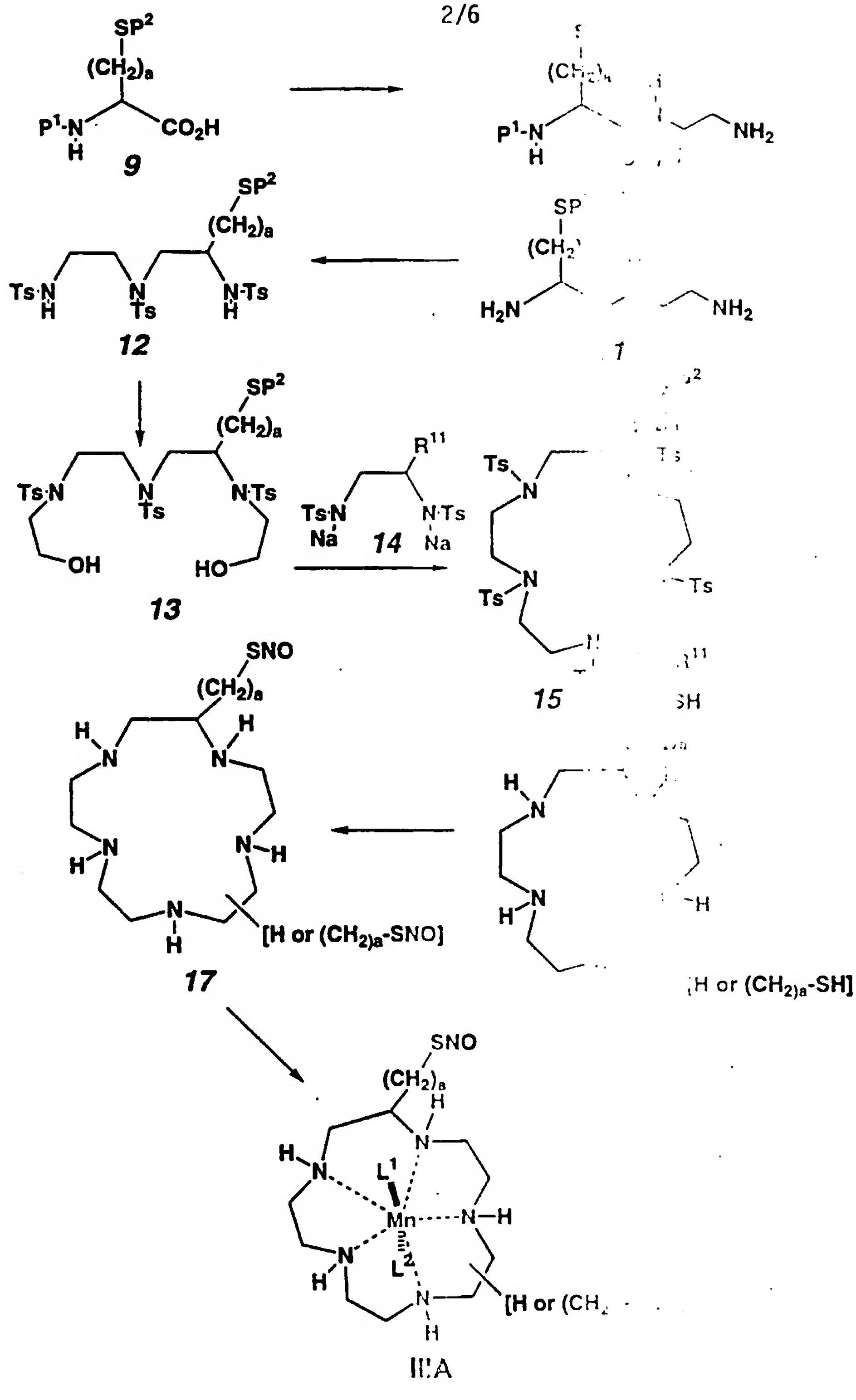
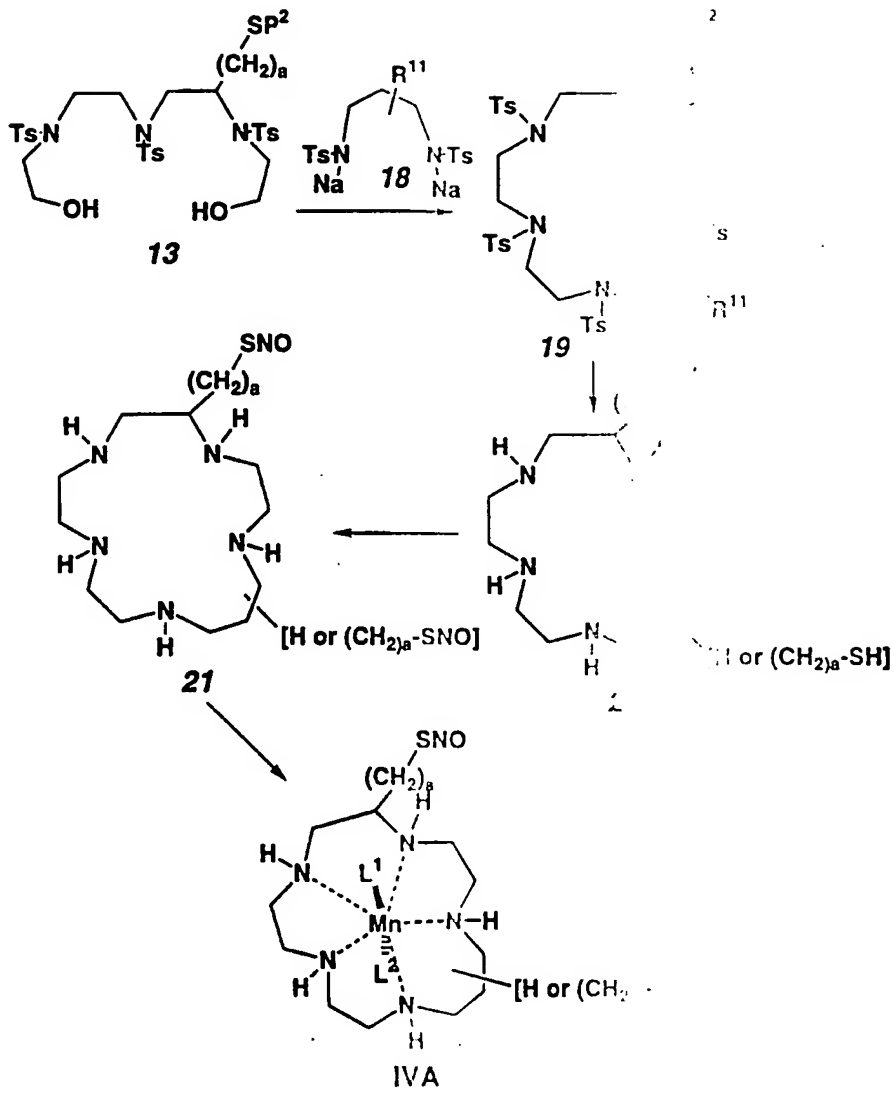


Figure 3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(CH2)a
Ts-N
N
Ts
N-Ts
OH
HO
13
R11
Ts-N
Na
18
N-Ts
Na
Ts
N
Ts
N
N
Ts
19
SNO
(CH2)a
H
N
H
N
H
N
N
H
N
H
[H or (CH2)a-SNO]
21
H
N
H
N
N
H
[H or (CH2)a-SH]
SNO
(CH2)a
H
N
H-N
L1
Mn
N-H
N
H
L2
N
H
[H or (CH2
IVA

Figure 4

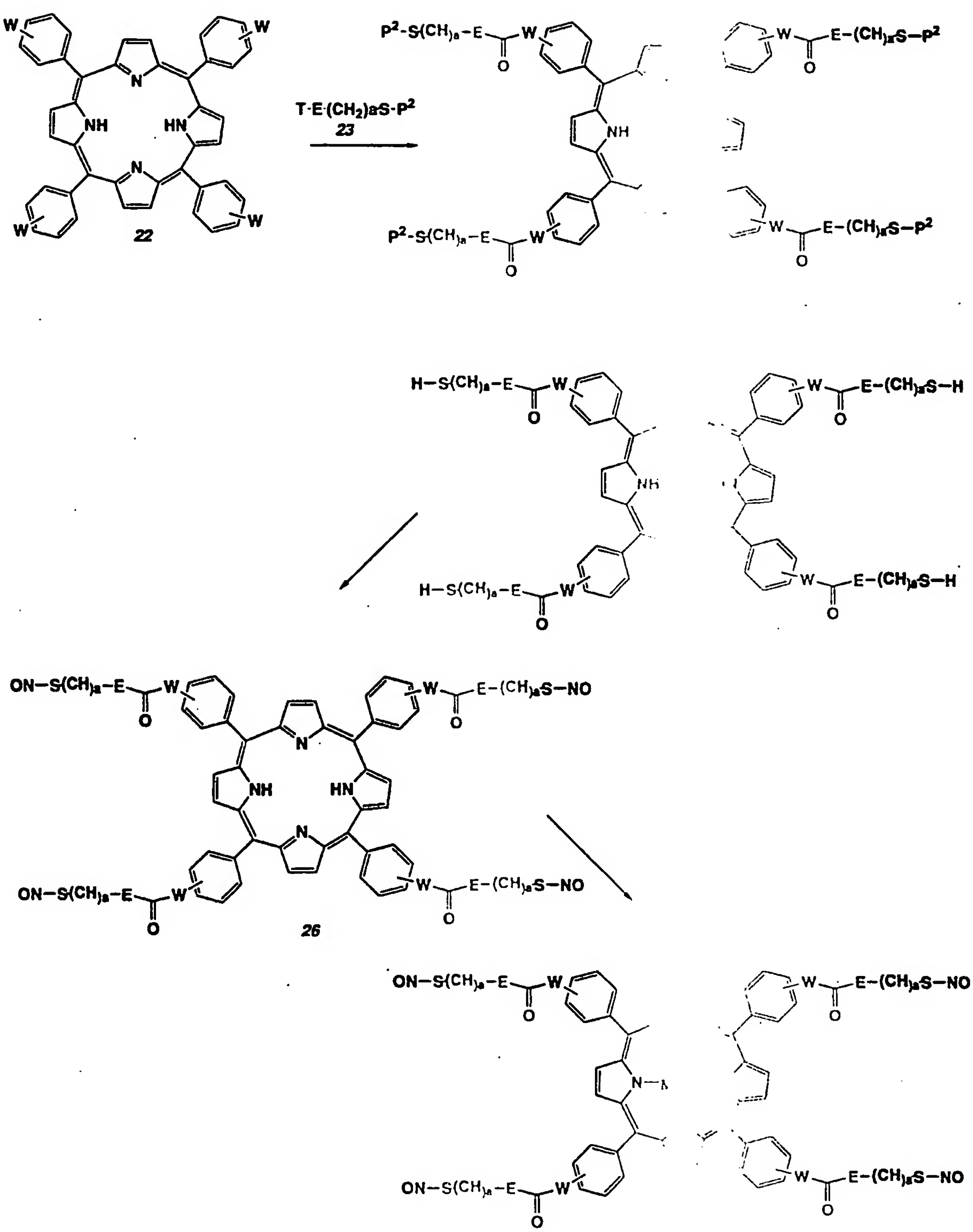
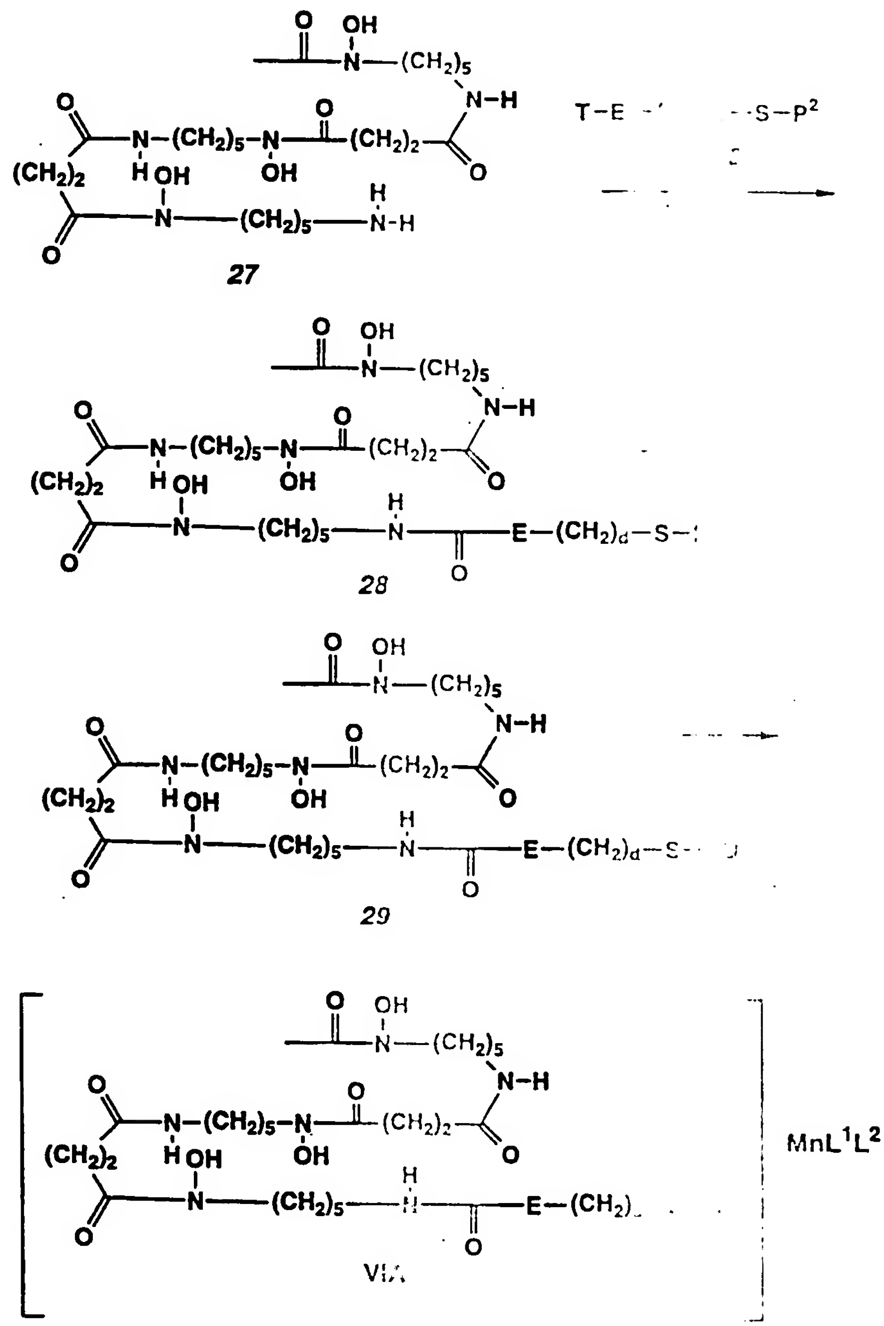


Figure 5



27

28

29

VIA

Figure 6

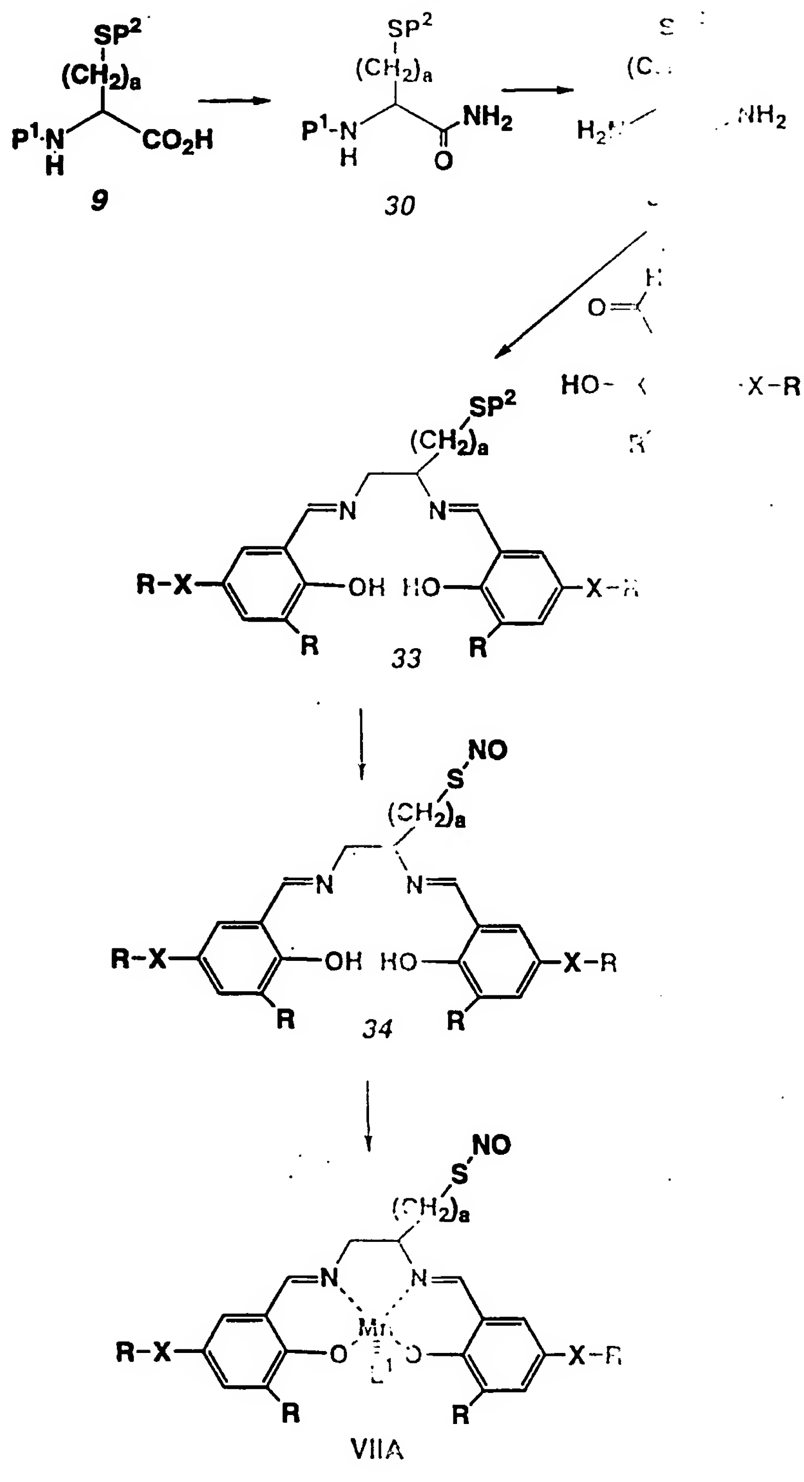

SP2
(CH2)a
P1-N
H
CO2H
9
SP2
(CH2)a
P1-N
H
NH2
O
30
NH2
HO-
X-R
SP2
(CH2)a
N
N
R-X
OH
HO
R
33
R
NO
S
(CH2)a
N
N
R-X
OH
HO
X-R
R
34
R
NO
S
N
N
R-X
O
R
VIIA
R

Fig: re 7

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/08406

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C07D 487/22; A61K 31/40
US CL :540/145; 514/185, 410; 548/400
According to International Patent Classification (IPC) or to both national classification an

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification sym
U.S. : 540/145; 514/185, 410; 548/400

Documentation searched other than minimum documentation to the extent that such docume ...cluded in the fields searched

Electronic data base consulted during the international search (name of data base and, whe ...cable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	US, A, 5,430,051 (AIZAWA ET AL.) 04 July ... see entire document.	3, 5-7
Y	US, A, 4,298,502 (HALLING ET AL.) 29 March ... see entire document.	3, 5-7
Y	US, A, 5,368,841 (TRAUNER ET AL.) 29 Novem... 94, see entire document.	3, 5-7

☐ Further documents are listed in the continuation of Box C. ☐ See pat... ...x.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but l... ...n the priority date claimed
"T" later docum... ...e international filing date or priority date and not ... application but cited to understand the principle or ... the invention
"X" document ... ...nce; the claimed invention cannot be considered n... considered to involve an inventive step when the d... ...ne
"Y" document of ... ...ce; the claimed invention cannot be considered t... ...ventive step when the document is combined w... ...r such documents, such combination being obvi... ...in the art
"&" document m... ...atent family

Date of the actual completion of the international search	Date of mailing o... search report
22 AUGUST 1996	27 SE
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer P.K. SRIPAI Telephone No.

Form PCT/ISA/210 (second sheet)(July 1992)*

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHAB

2. Where no meaningful search could be carried out, specifically:

Claims 1, 2 and 10 are drawn to "compound". One does not know which 'com ... ed. It is not possible to classify the 'compound' without knowing the chemical structure; thus rendering ... e. The same reasoning applies to claims that are directly or indirectly dependent on claims 1 a ... ms 4 and 8-20, as well as claims 21-26 which refer thereto. The claims have been searched to the ... d 5-7.